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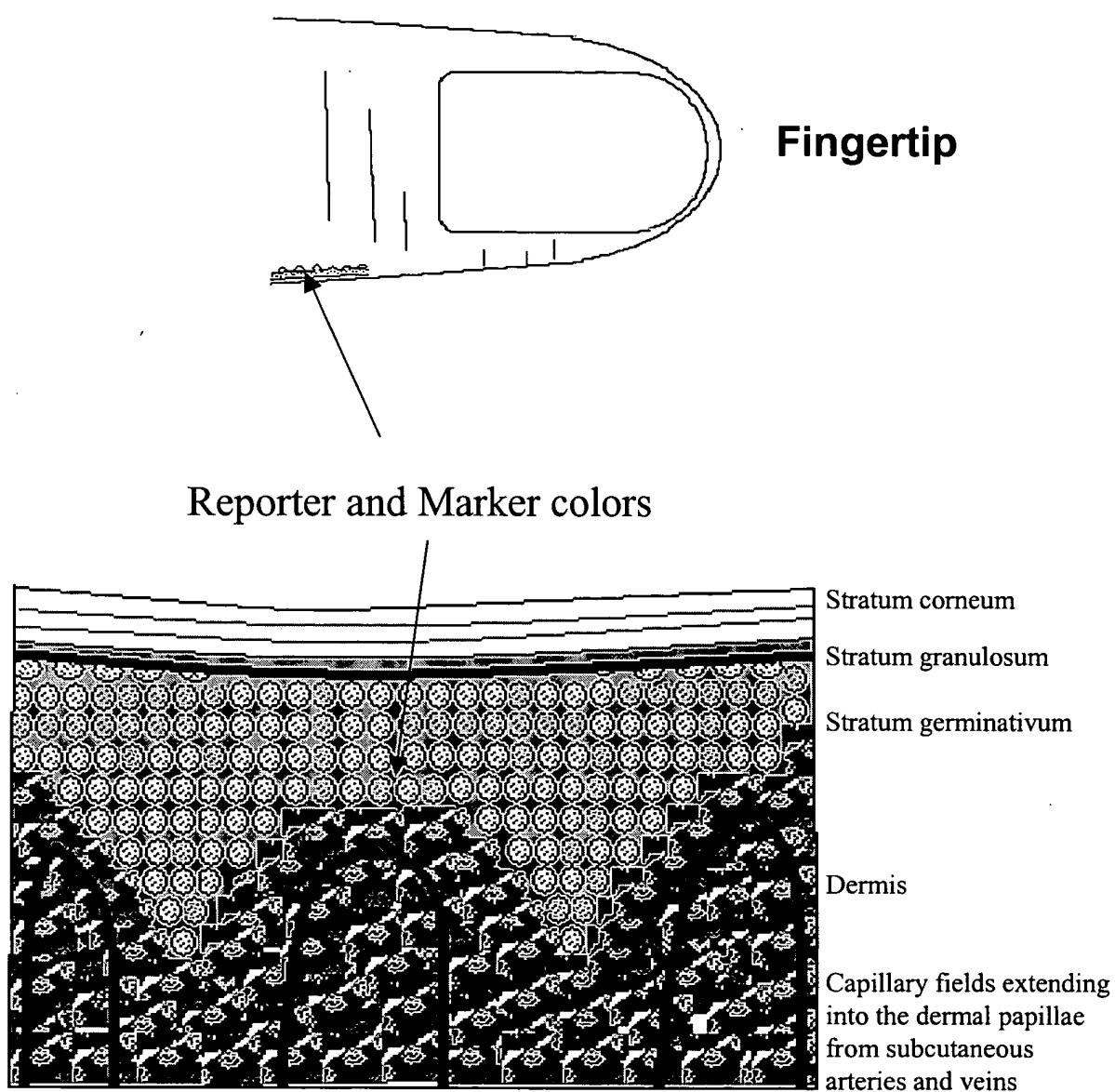


FIG. 1

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FIG. 2A

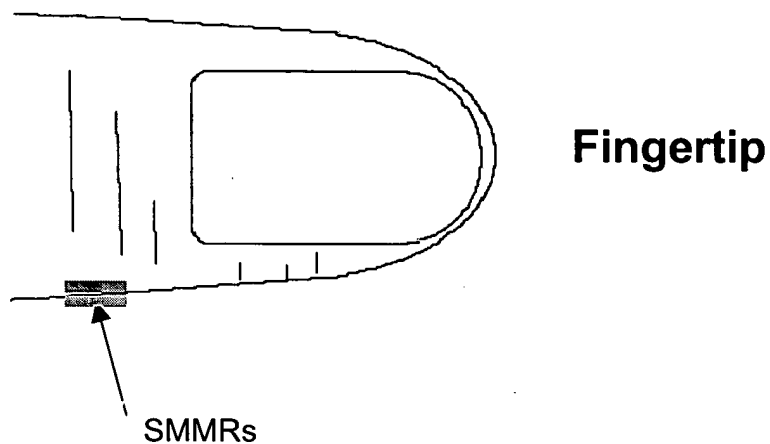
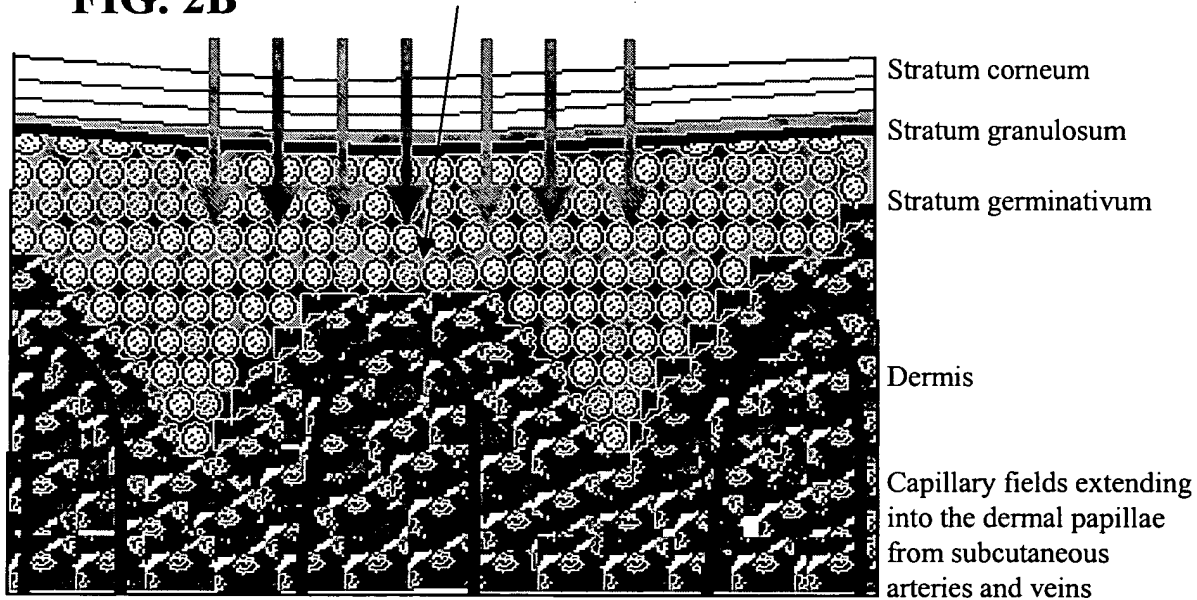


FIG. 2B



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FIG. 3A

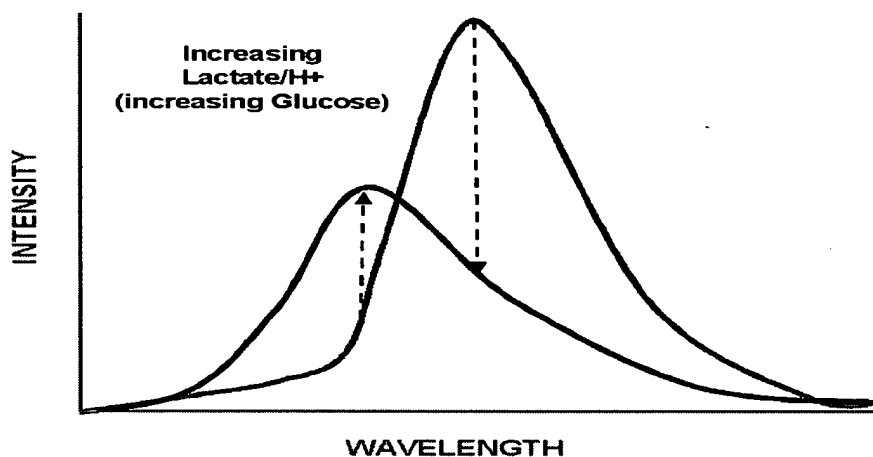
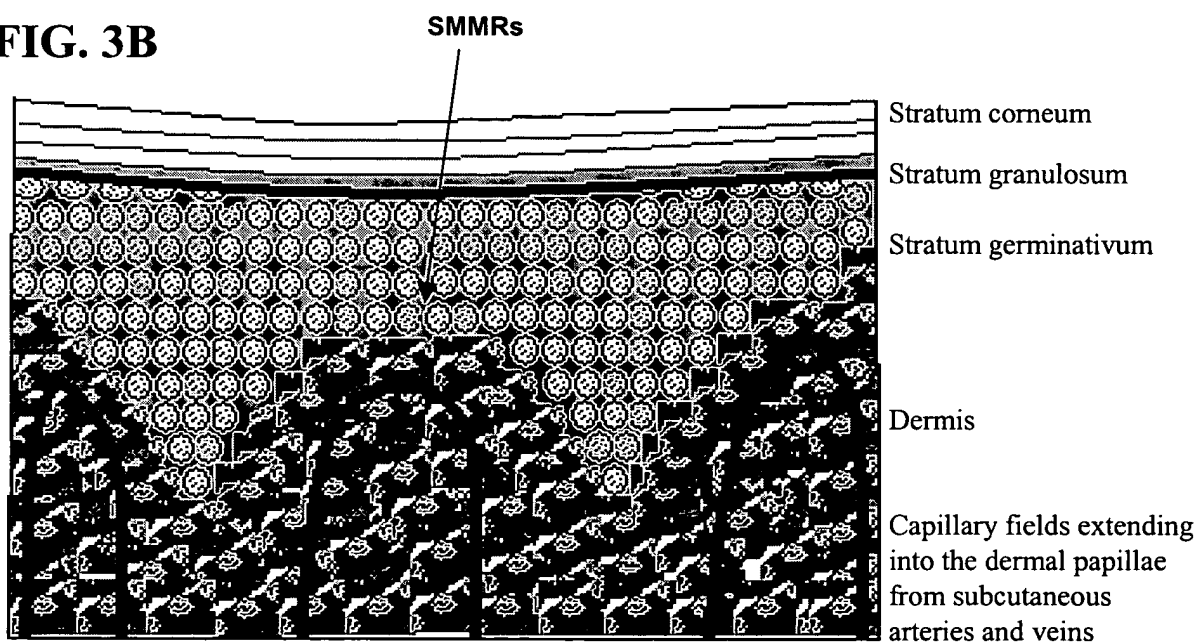


FIG. 3B



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FIG. 4A

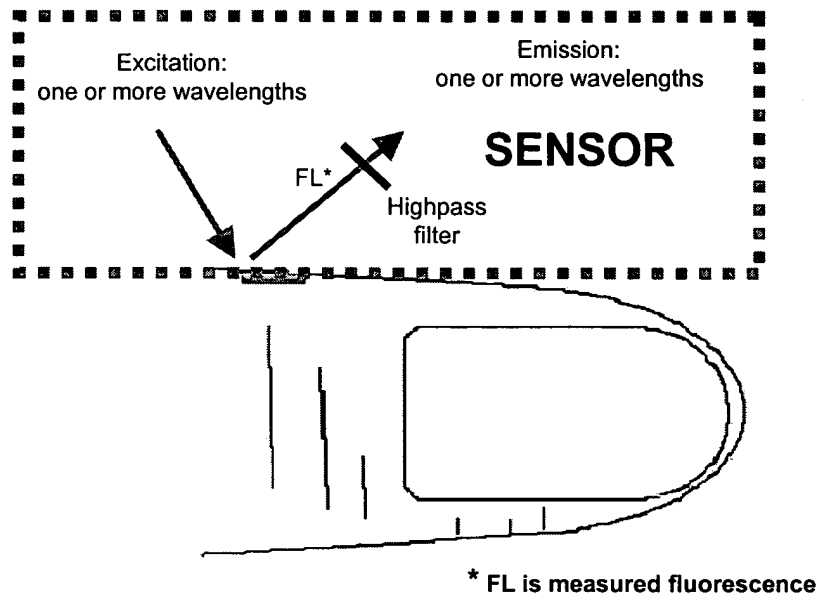
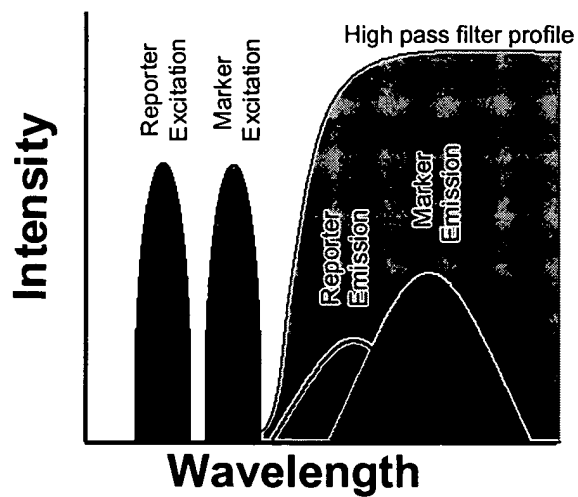


FIG. 4B



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FIG. 5A

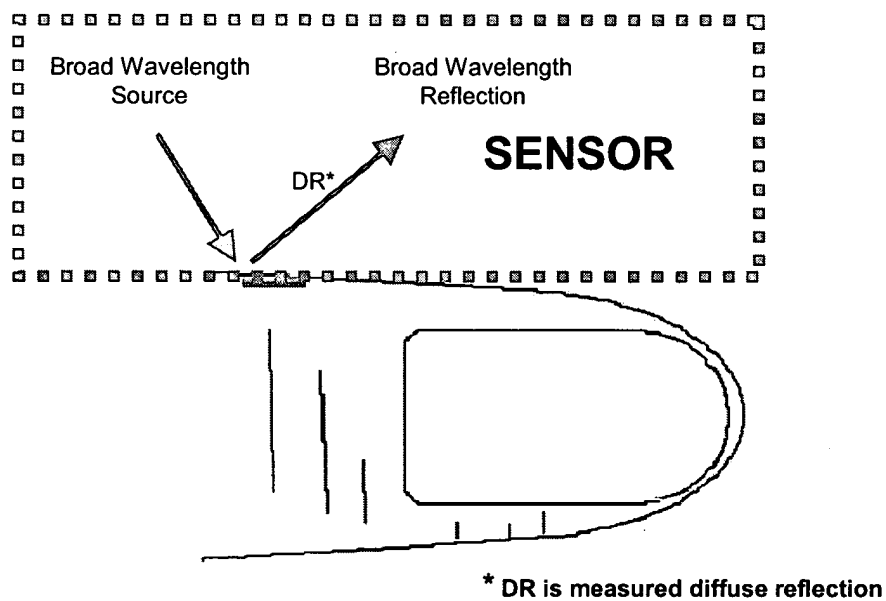
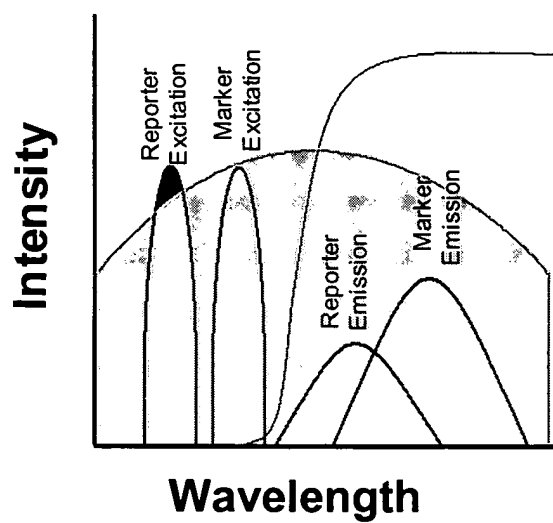


FIG. 5B



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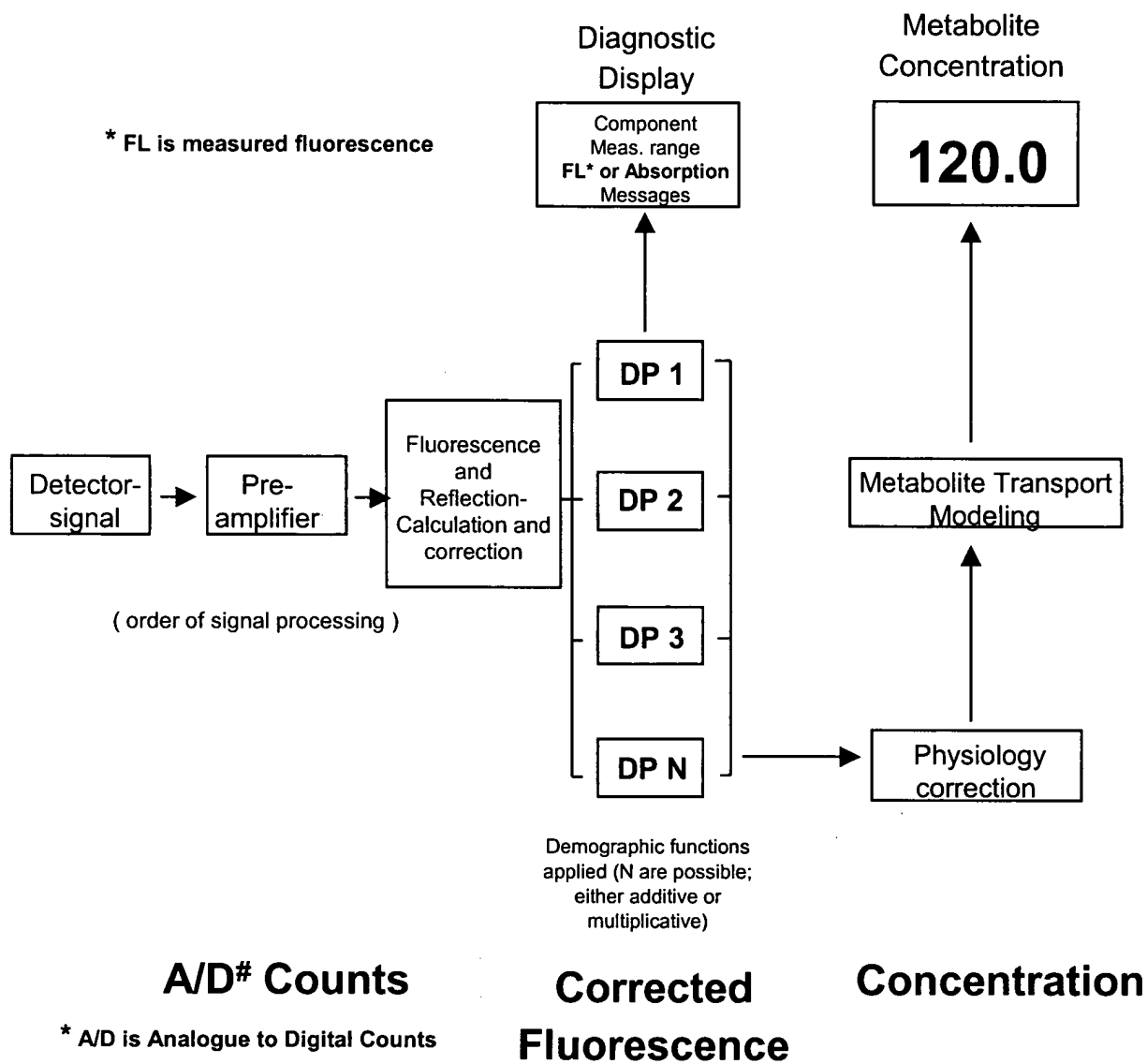


FIG. 6

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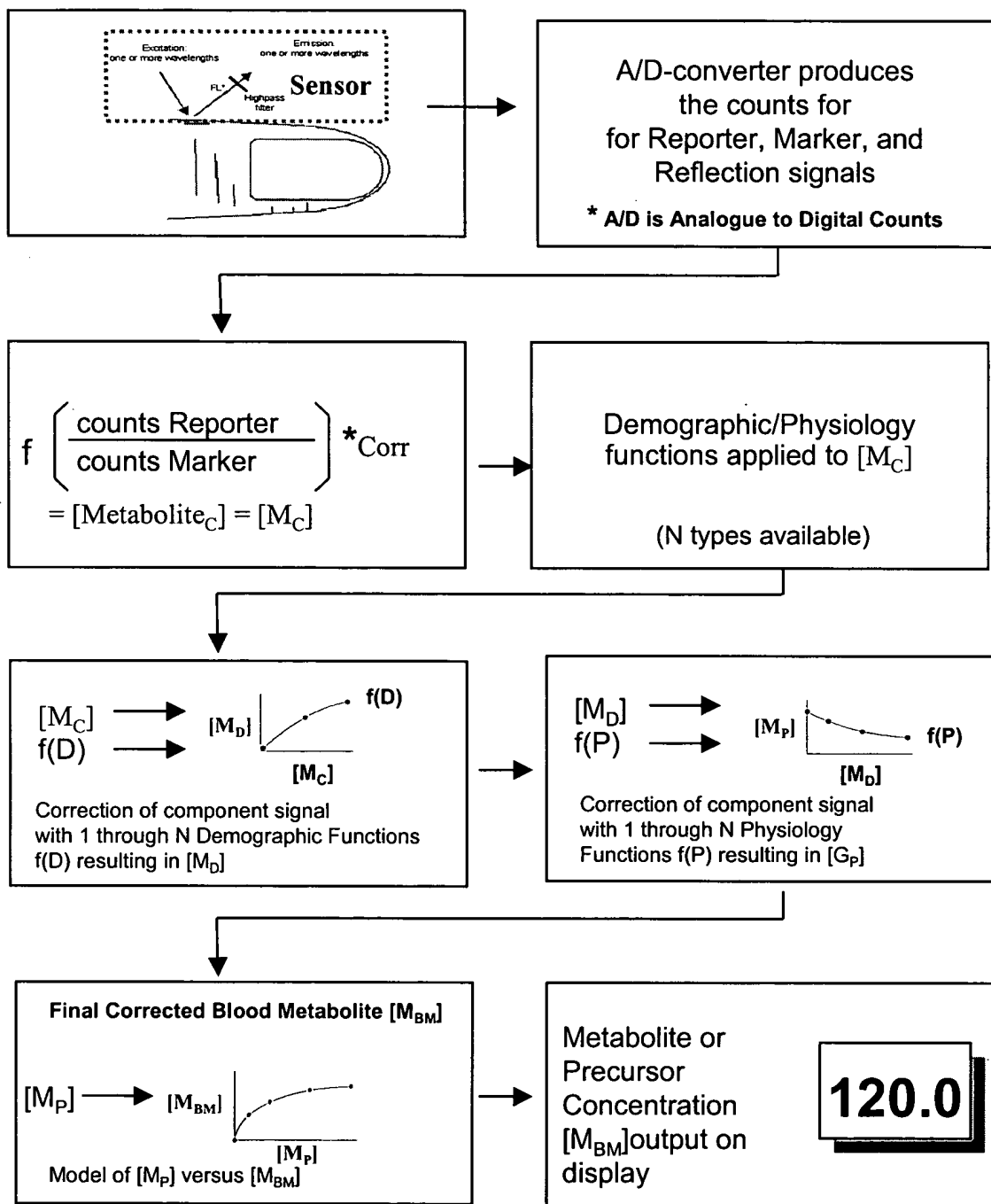


FIG. 7

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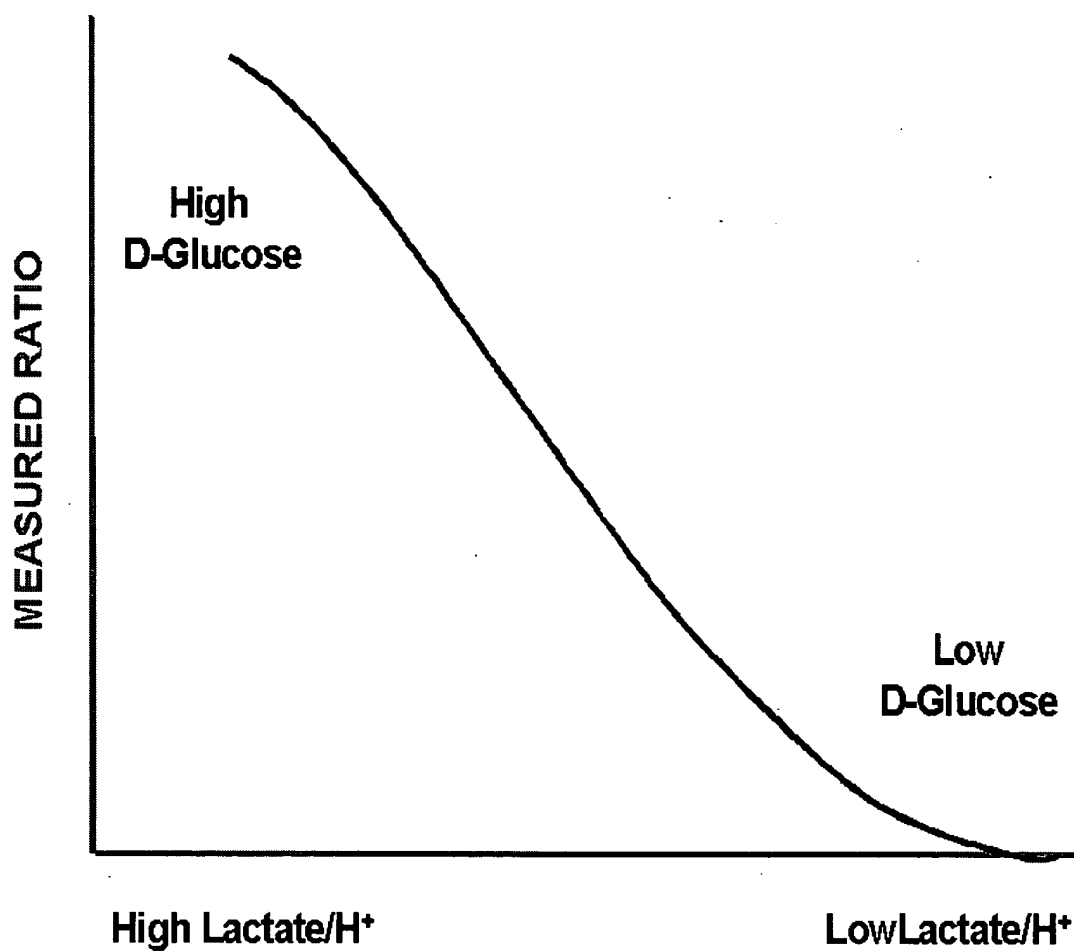


FIG. 8

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FIG. 9A

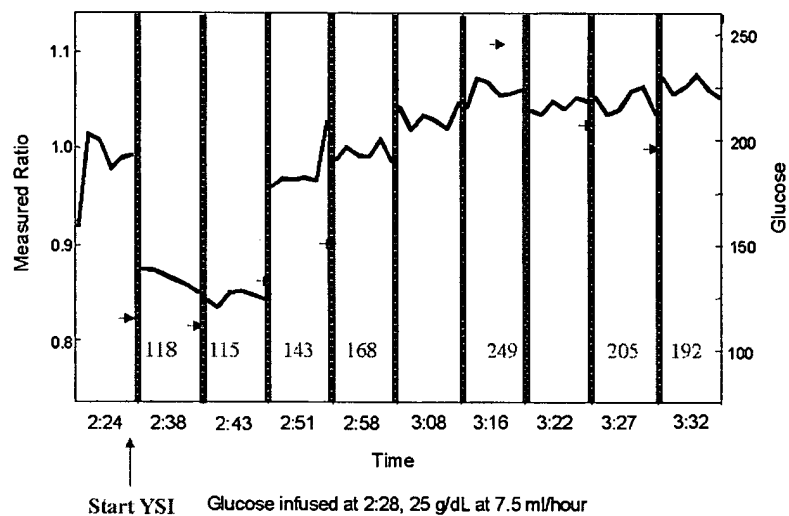
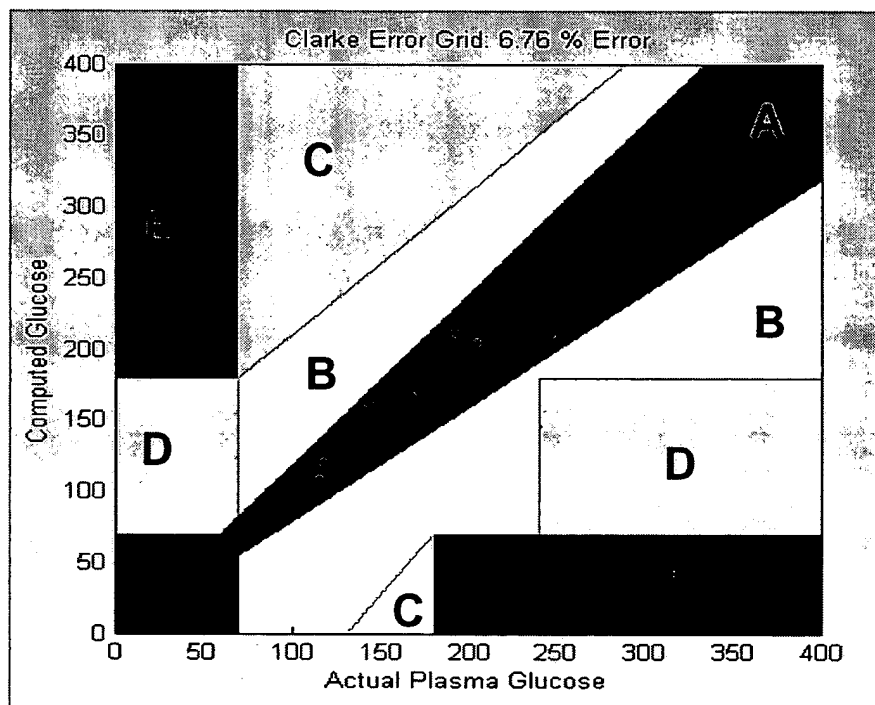
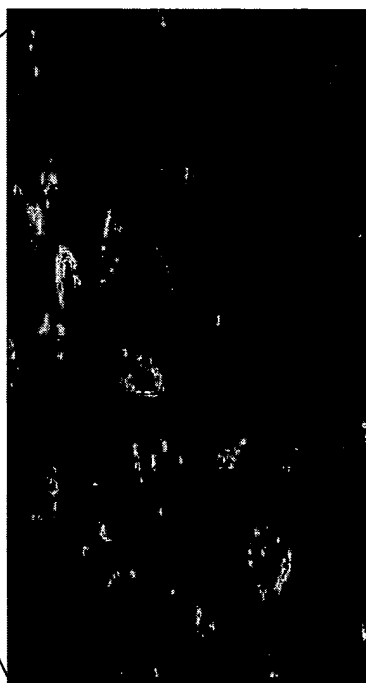


FIG. 9B



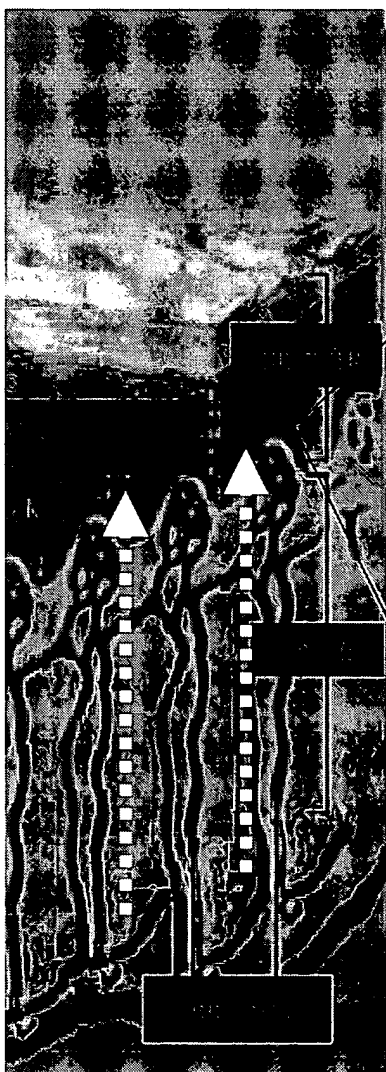
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**FIG.
10B**



Human keratinocytes

FIG. 10A



Human skin

Disposable patch added
to skin surface – passive
transport of SMMR to
keratinocytes

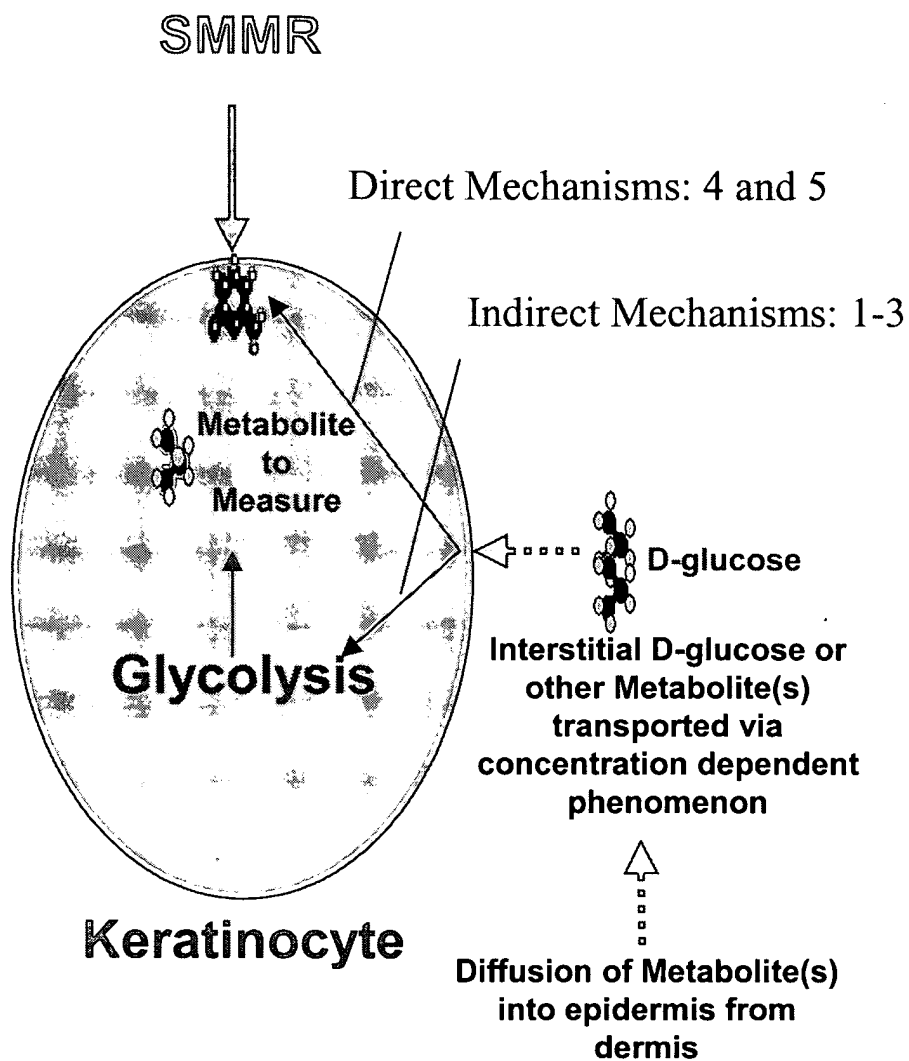


FIG. 11

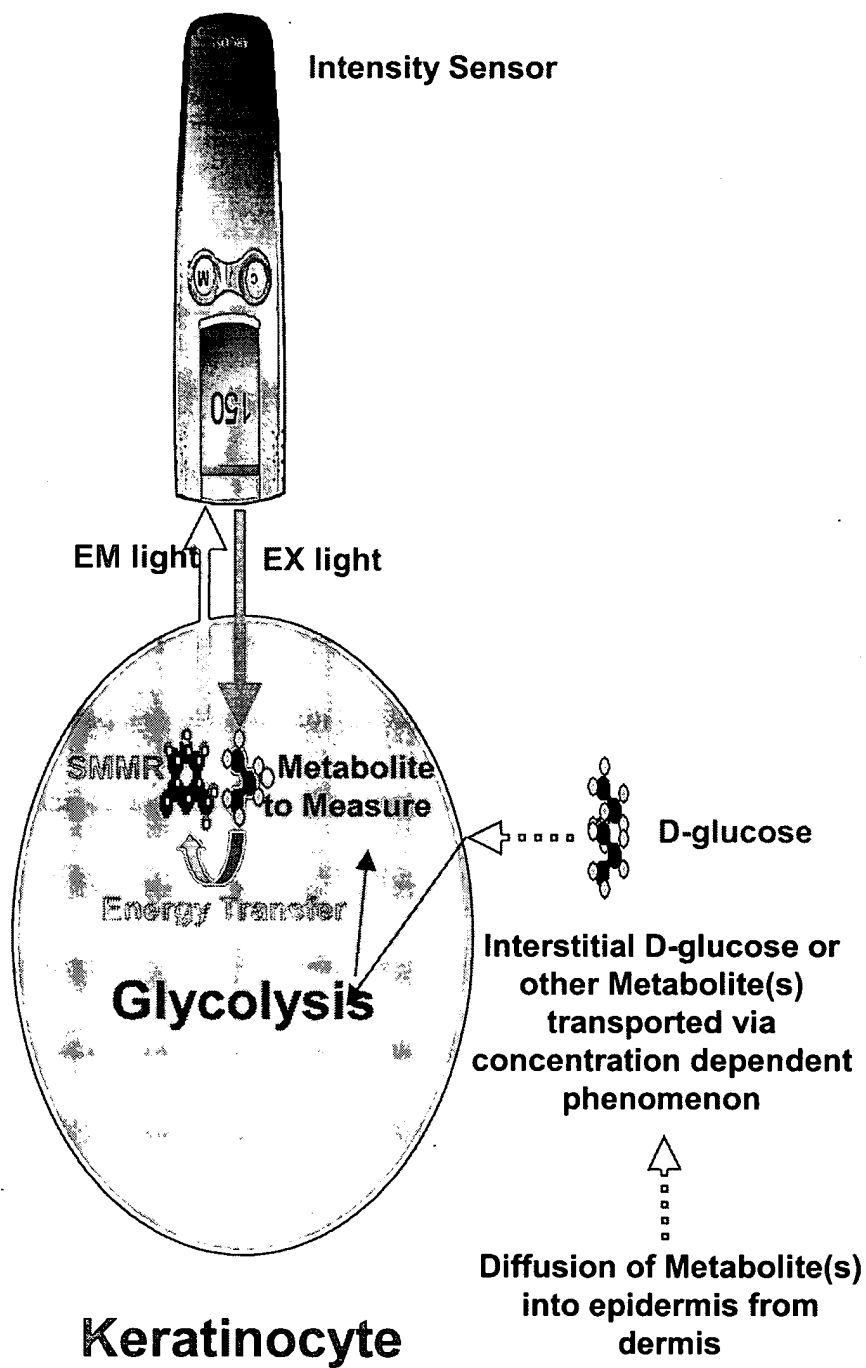


FIG. 12

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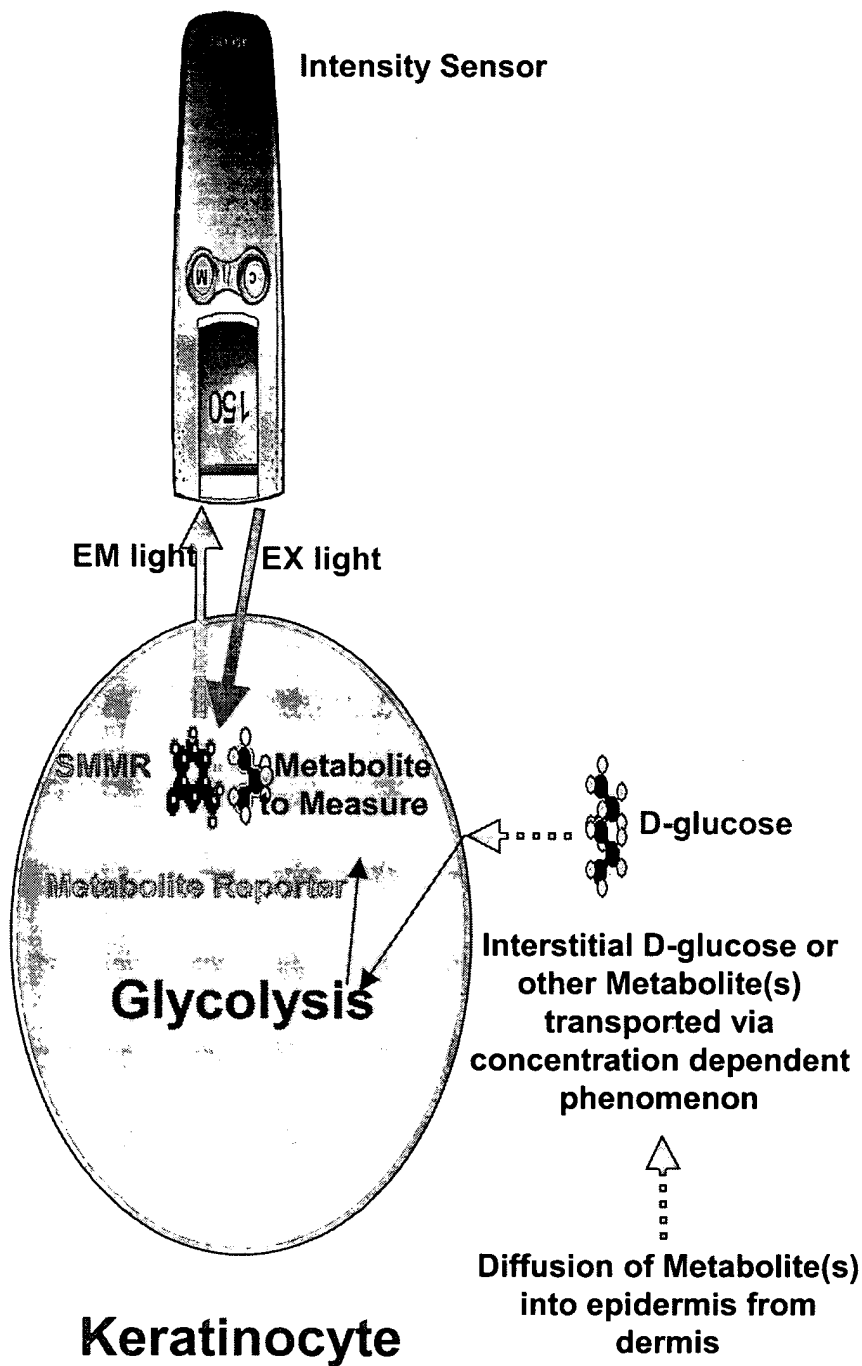


FIG. 13

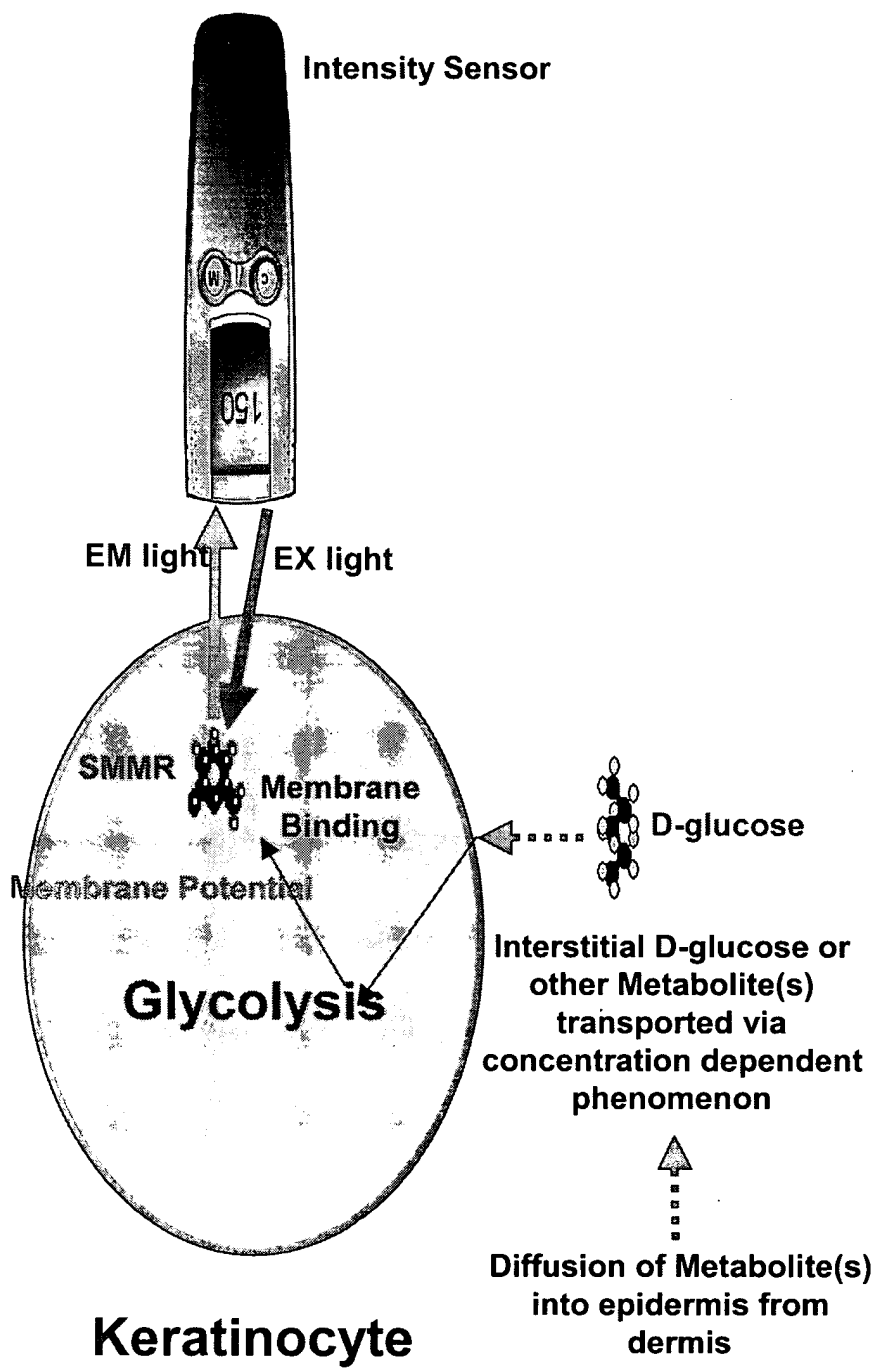


FIG. 14

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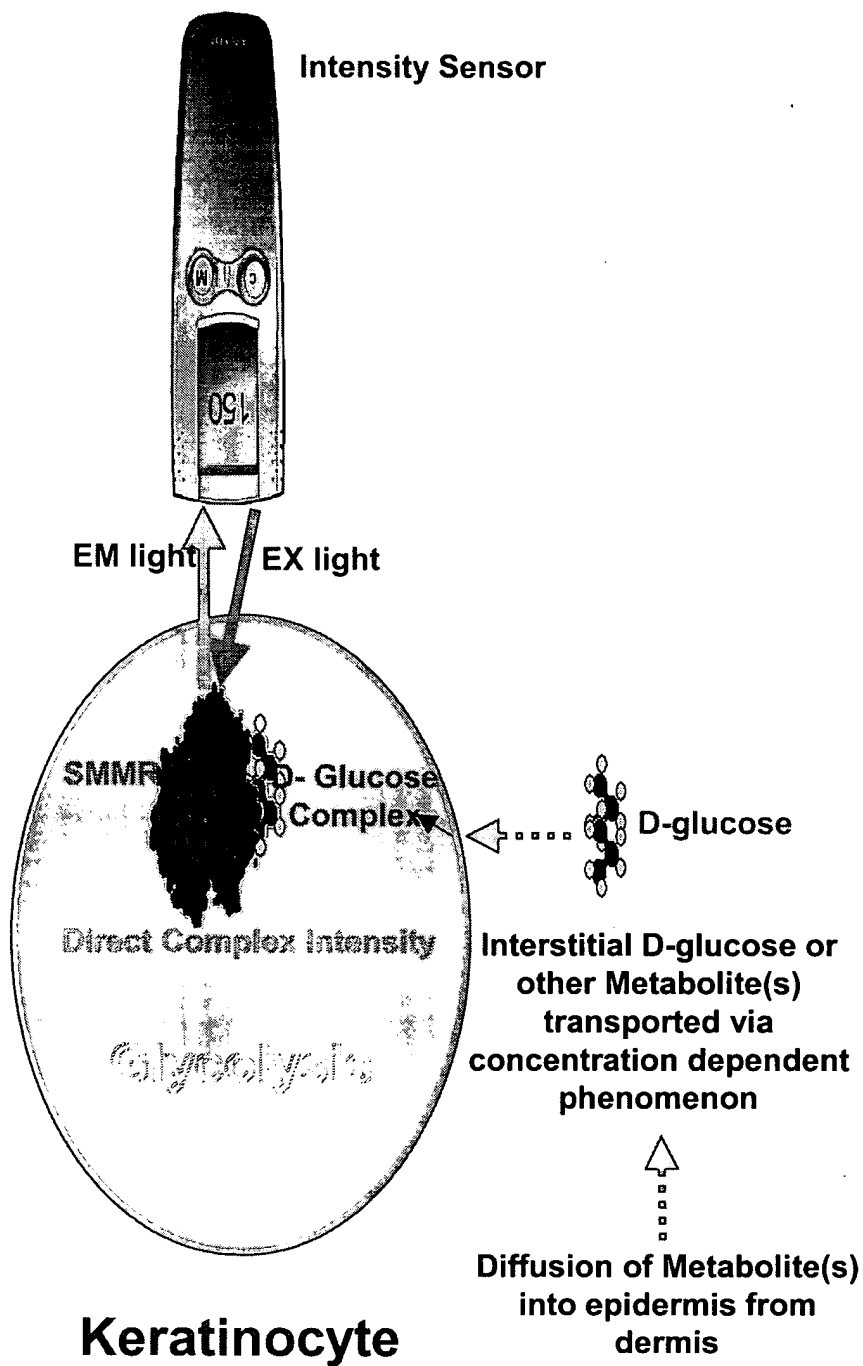


FIG. 15

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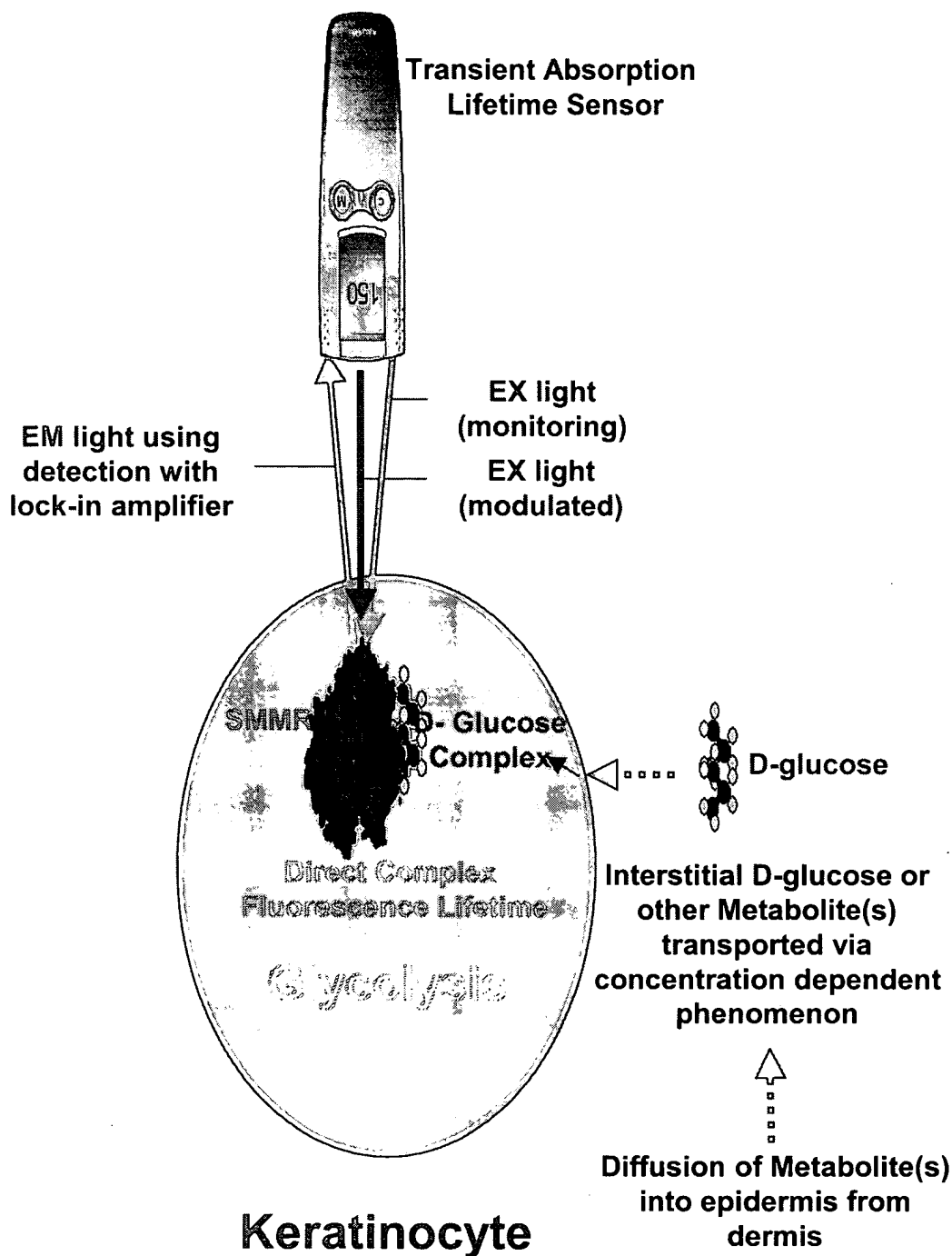
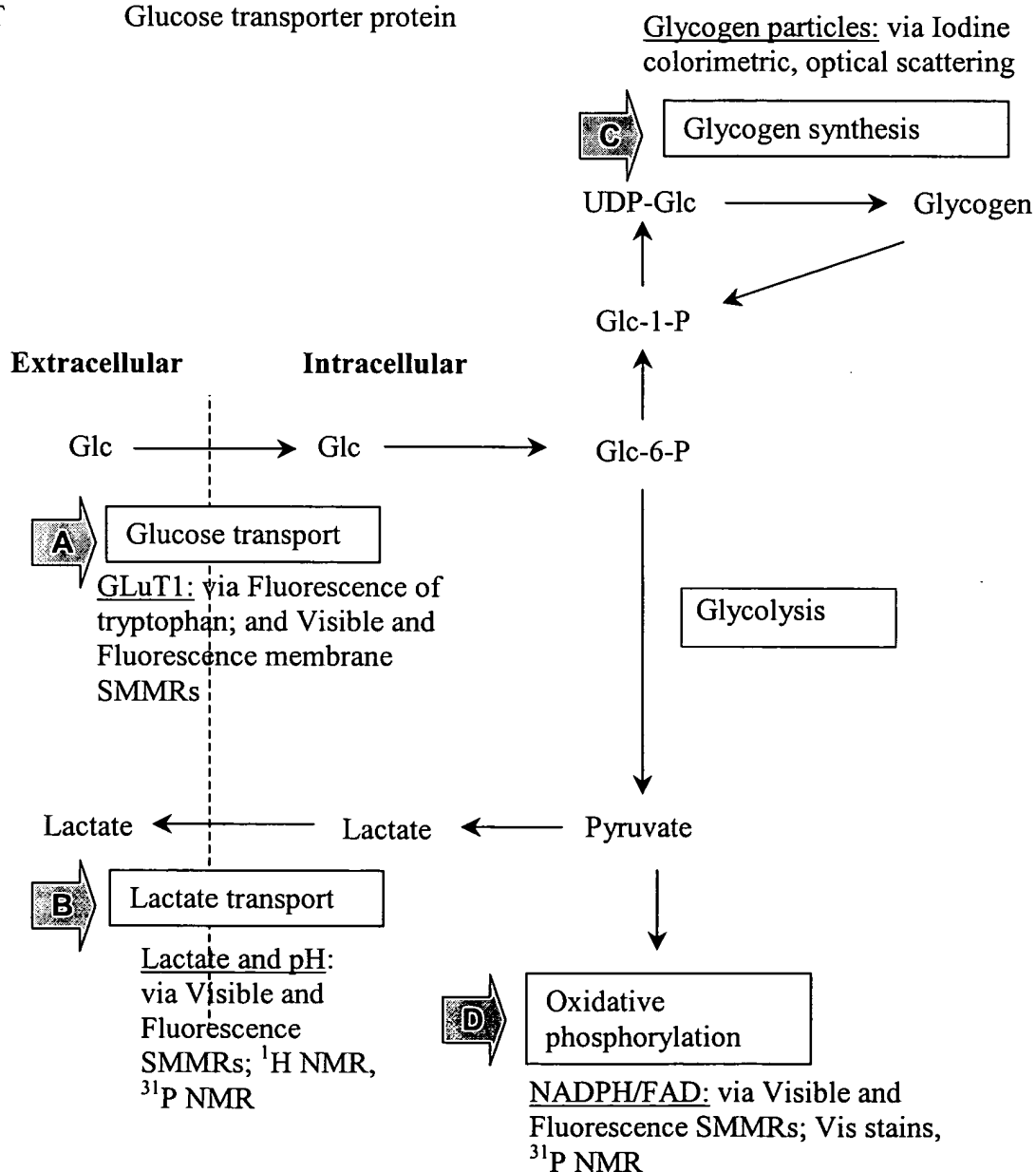


FIG. 16

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FIG. 17AScheme 1

Glc	Intracellular glucose
Pyr	Pyruvate
Glc-6-P	Glucose-6-phosphate
Glc-1-P	Glucose-1-phosphate
UDP-Glc	UDP-glucose
GluT	Glucose transporter protein



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FIG. 17B

Scheme 2. Overview of metabolic pathways for glucose in epidermis

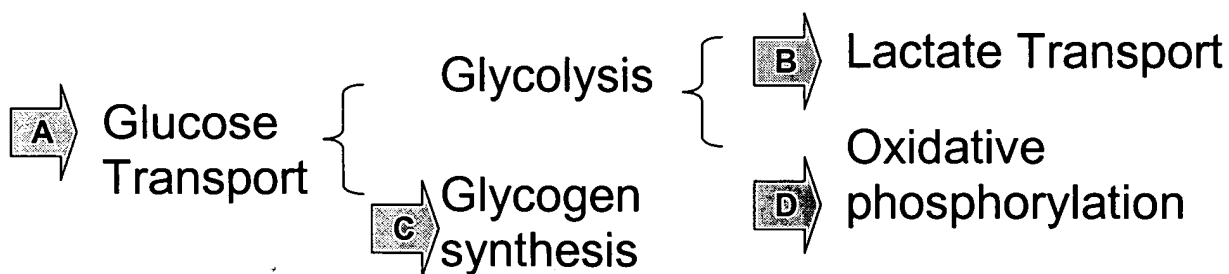
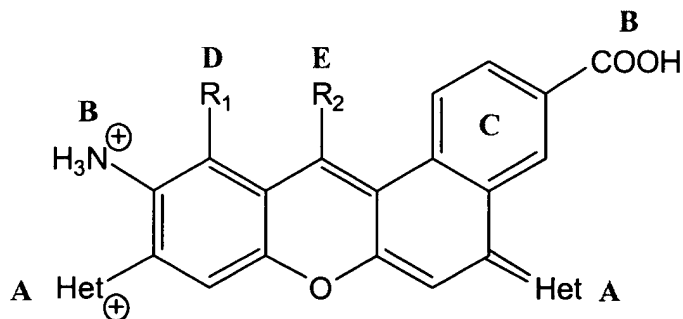
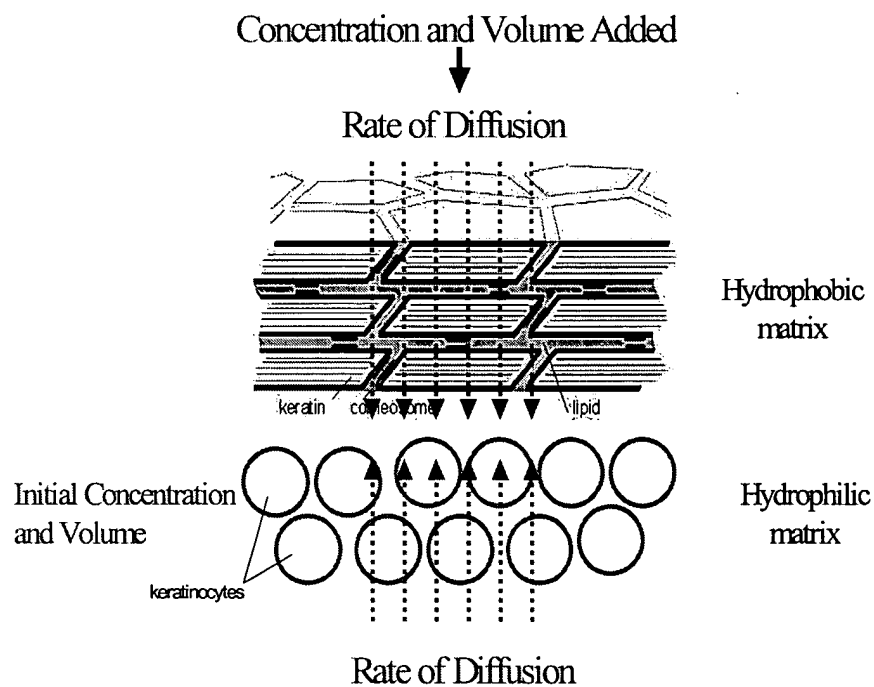


FIG. 17C

Scheme 3. Structure of generic pH sensitive dye for specific action as a lactate/H⁺ SMMR



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Scheme 4. *In Vivo* Calibration Issues

FIG. 17D

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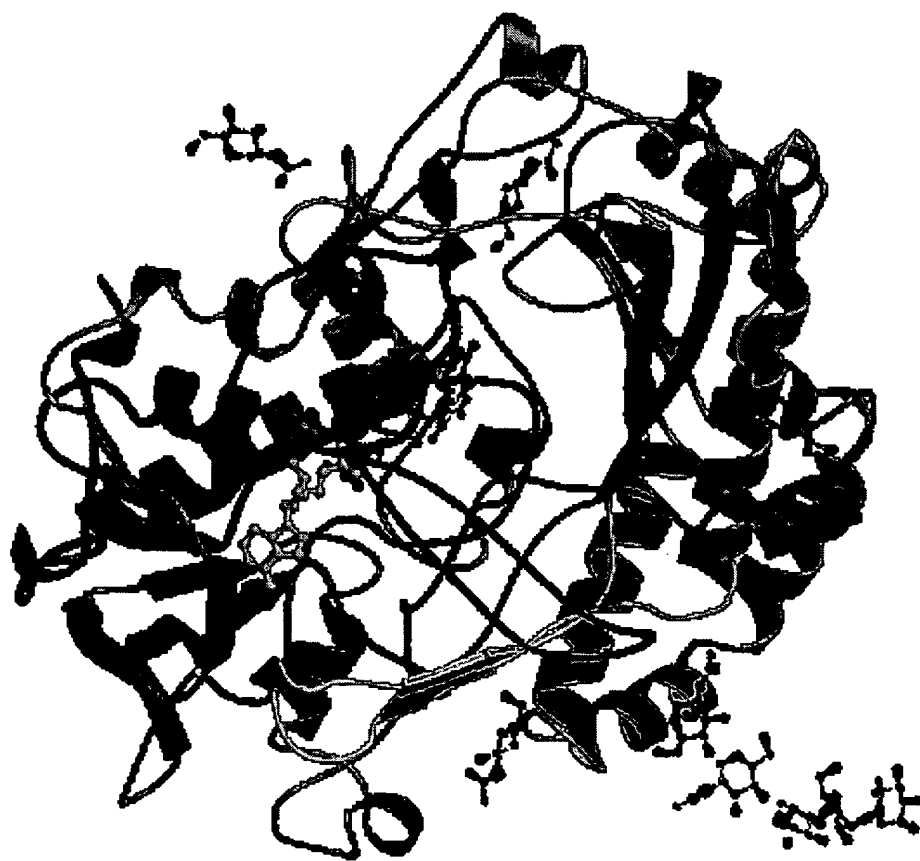


FIG. 18

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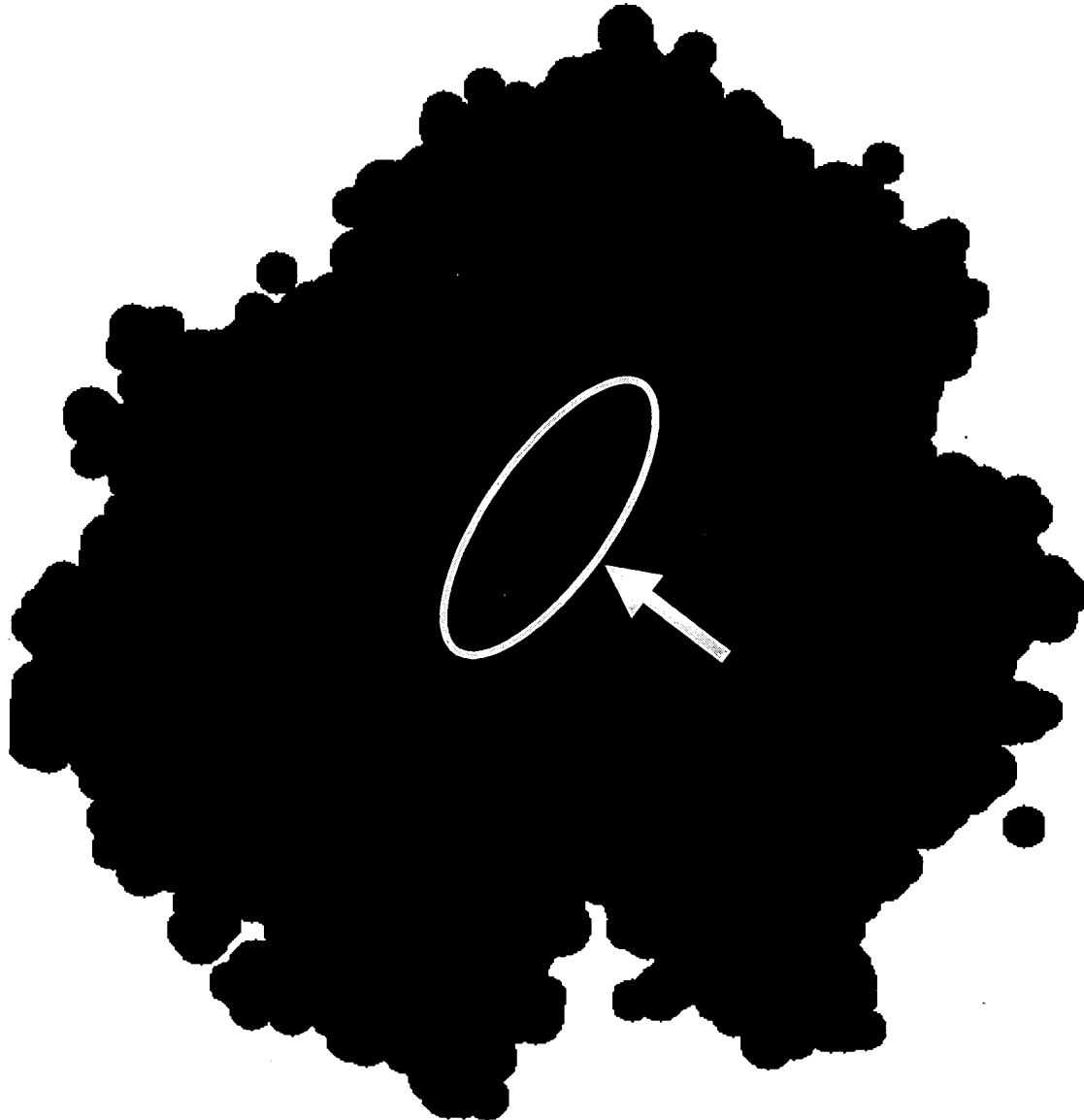


FIG. 19

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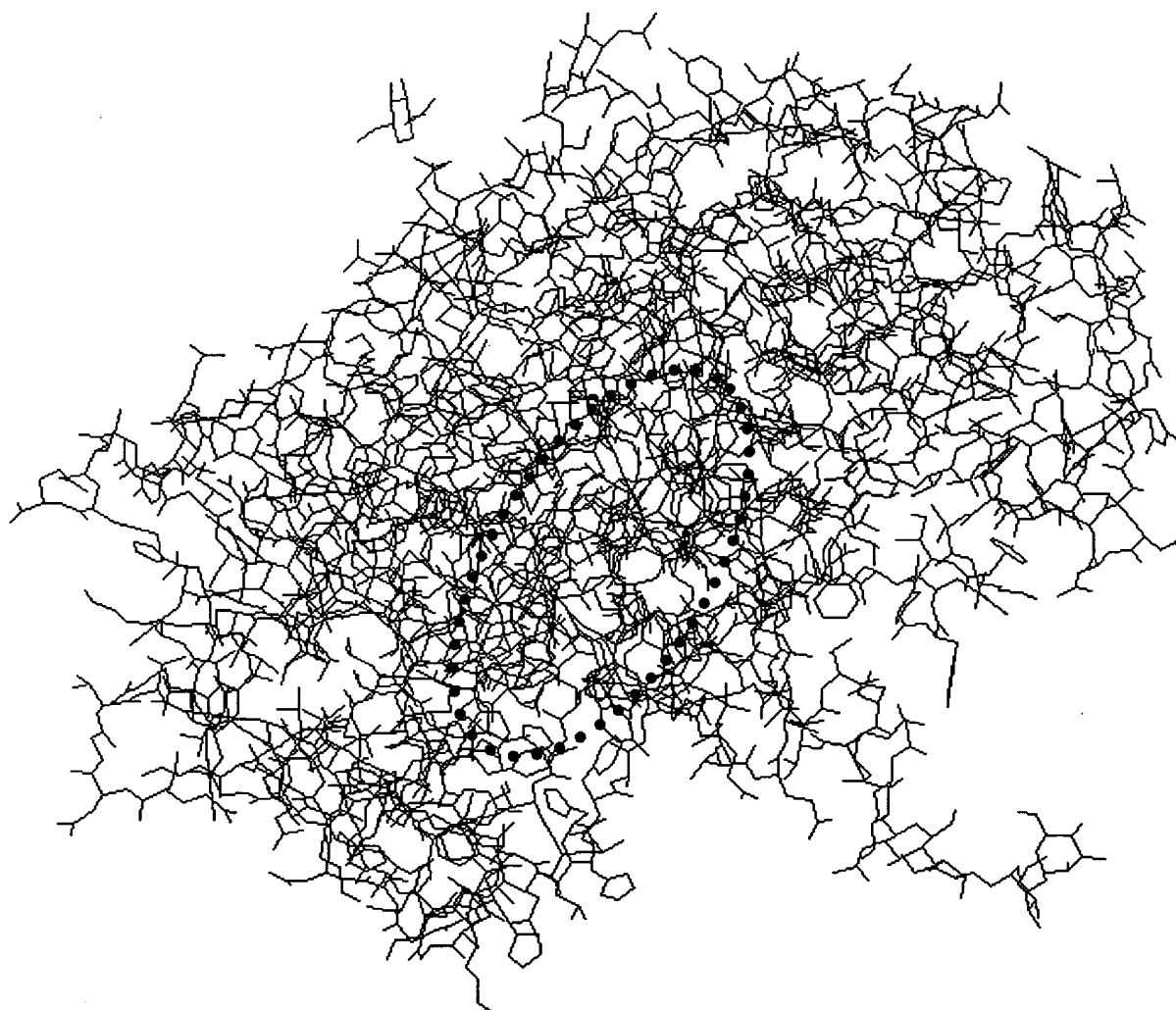


FIG. 20

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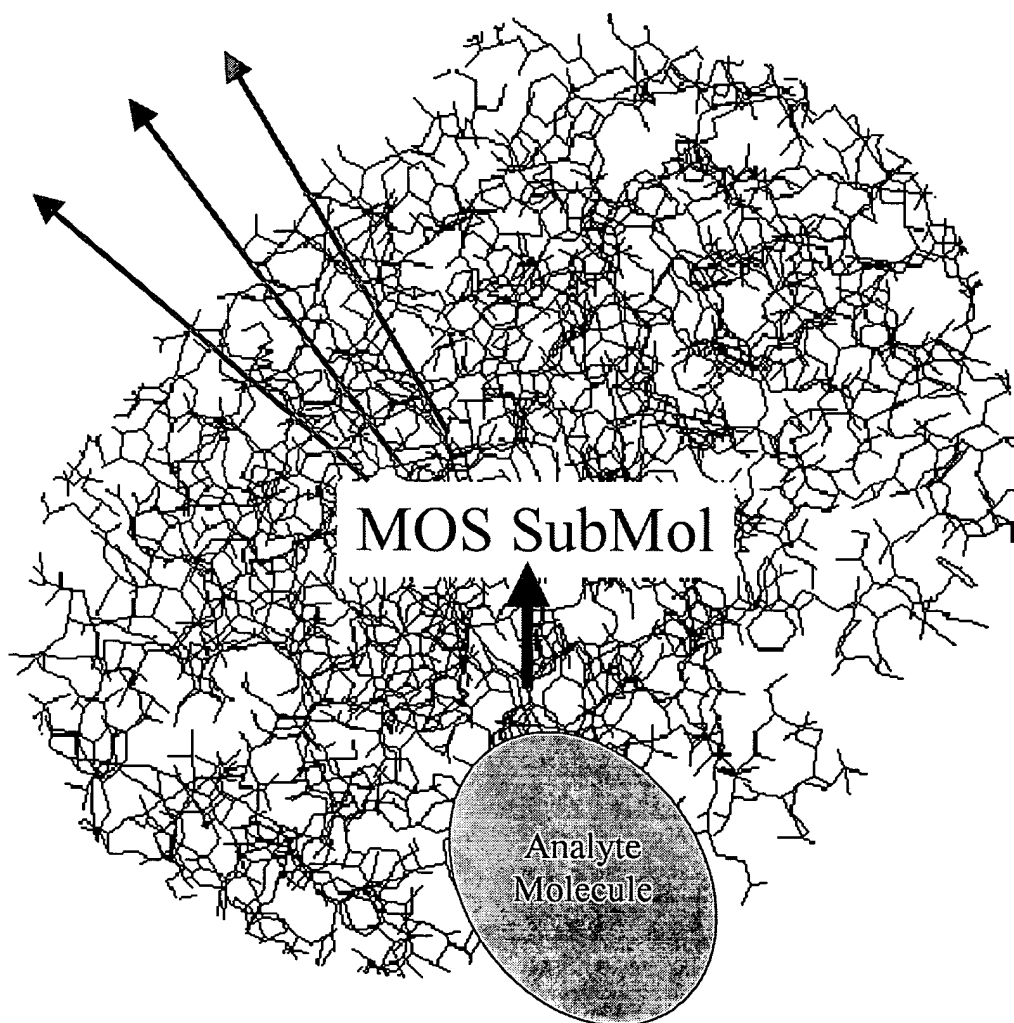


FIG. 21

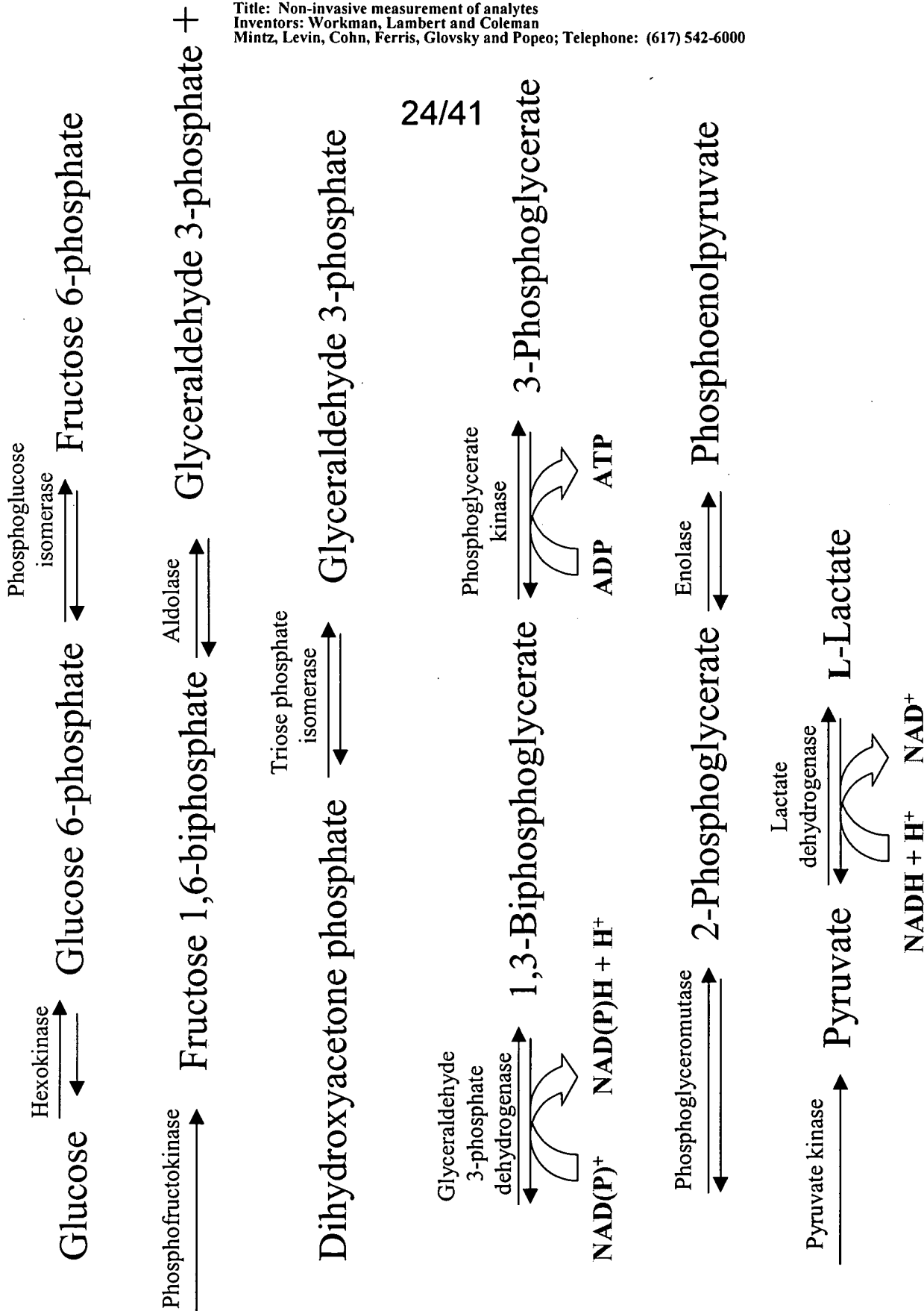
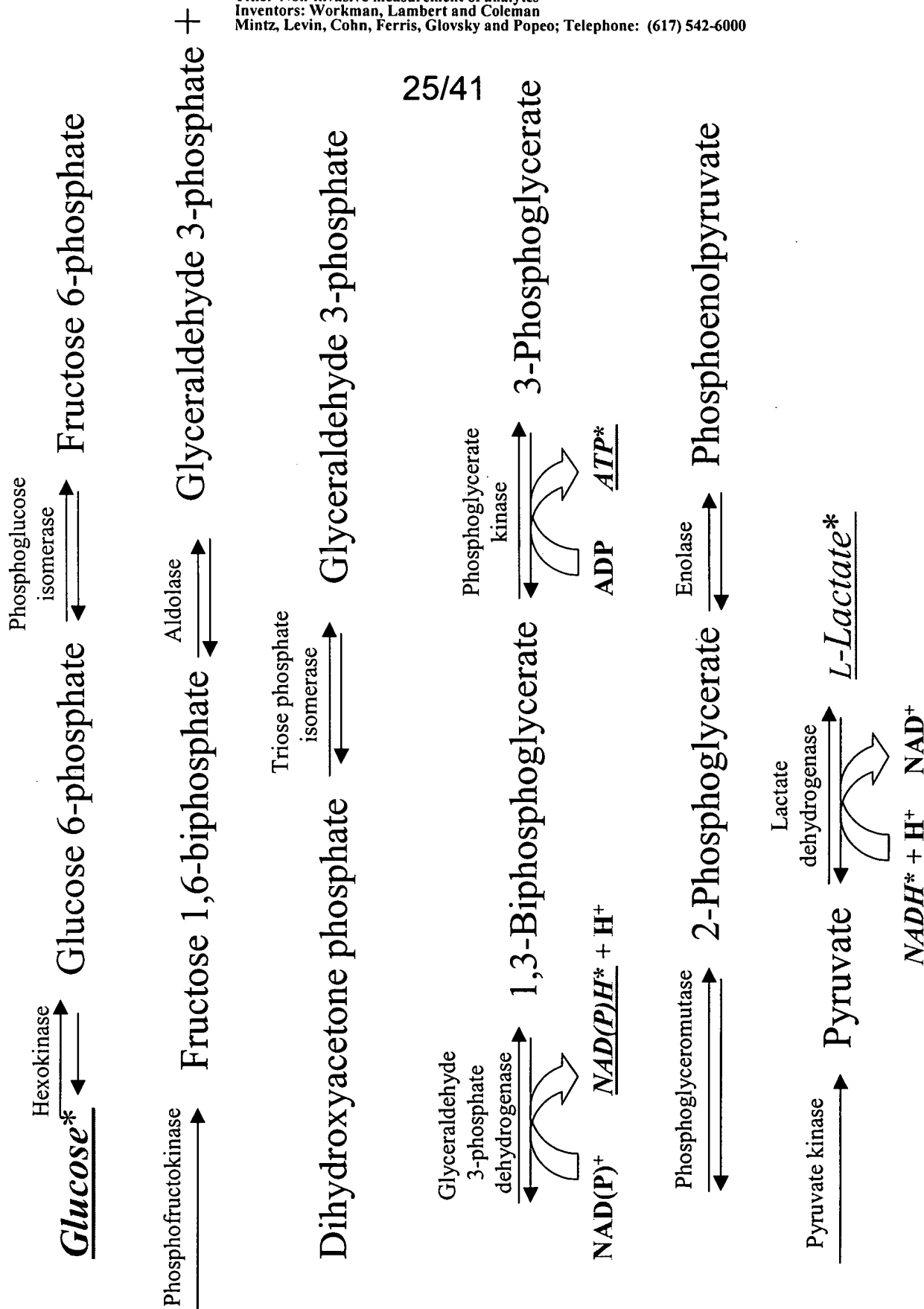


FIG. 22

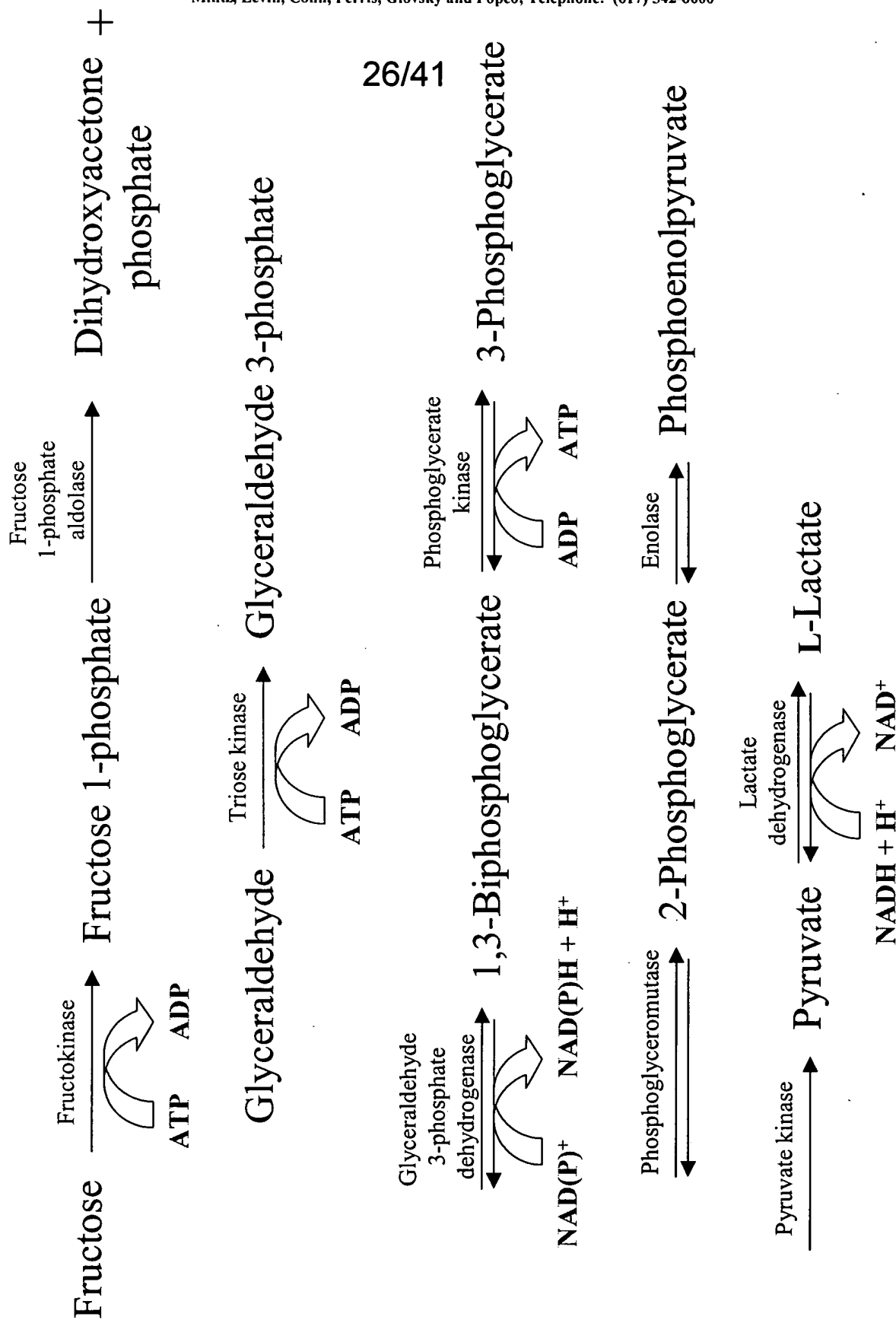
GLUCOSE GLYCOLYSIS

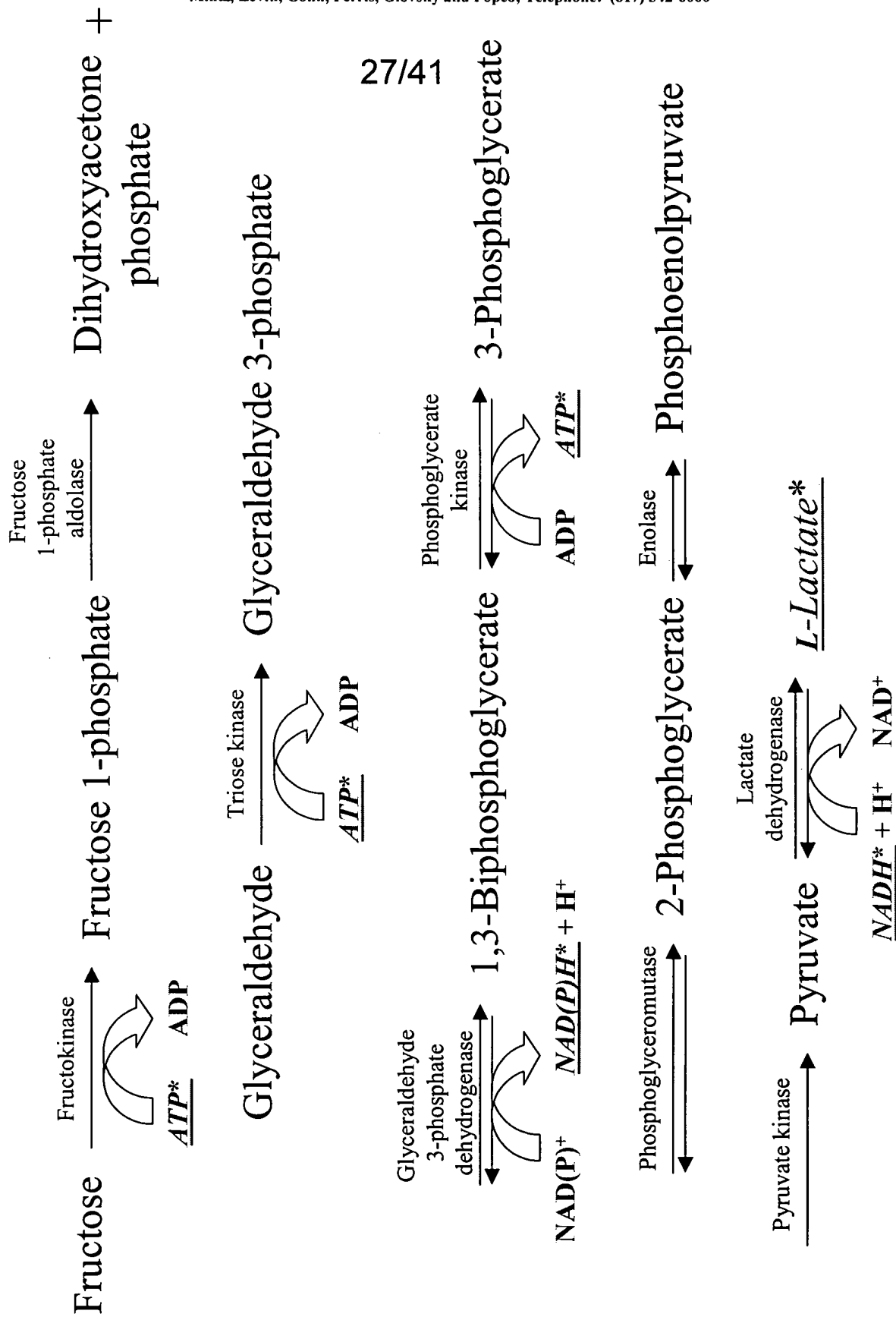


*Detectable Analytes (direct or indirect)

GLUCOSE GLYCOLYSIS

FIG. 23

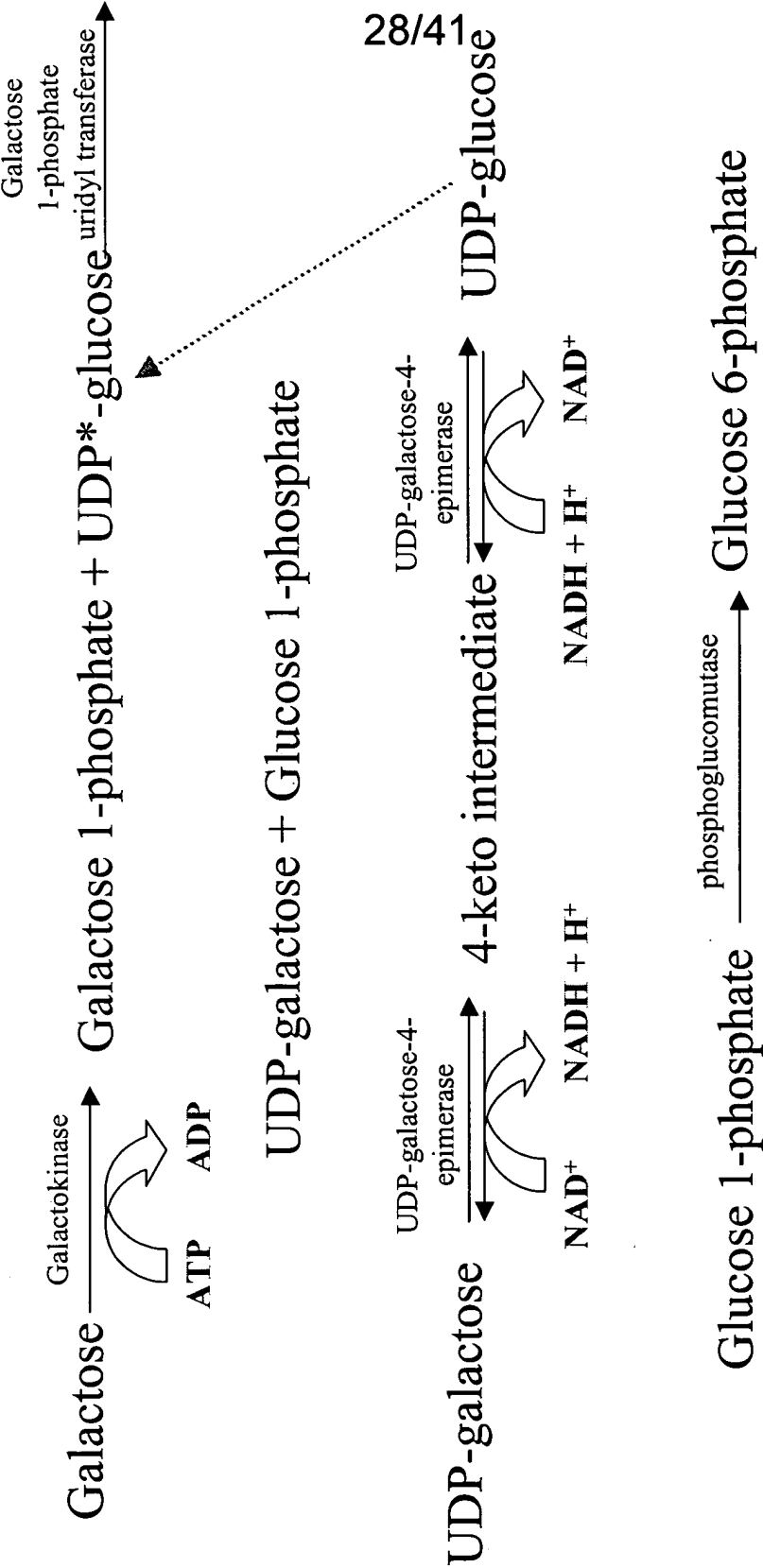




***Detectable Analytes (direct or indirect)**

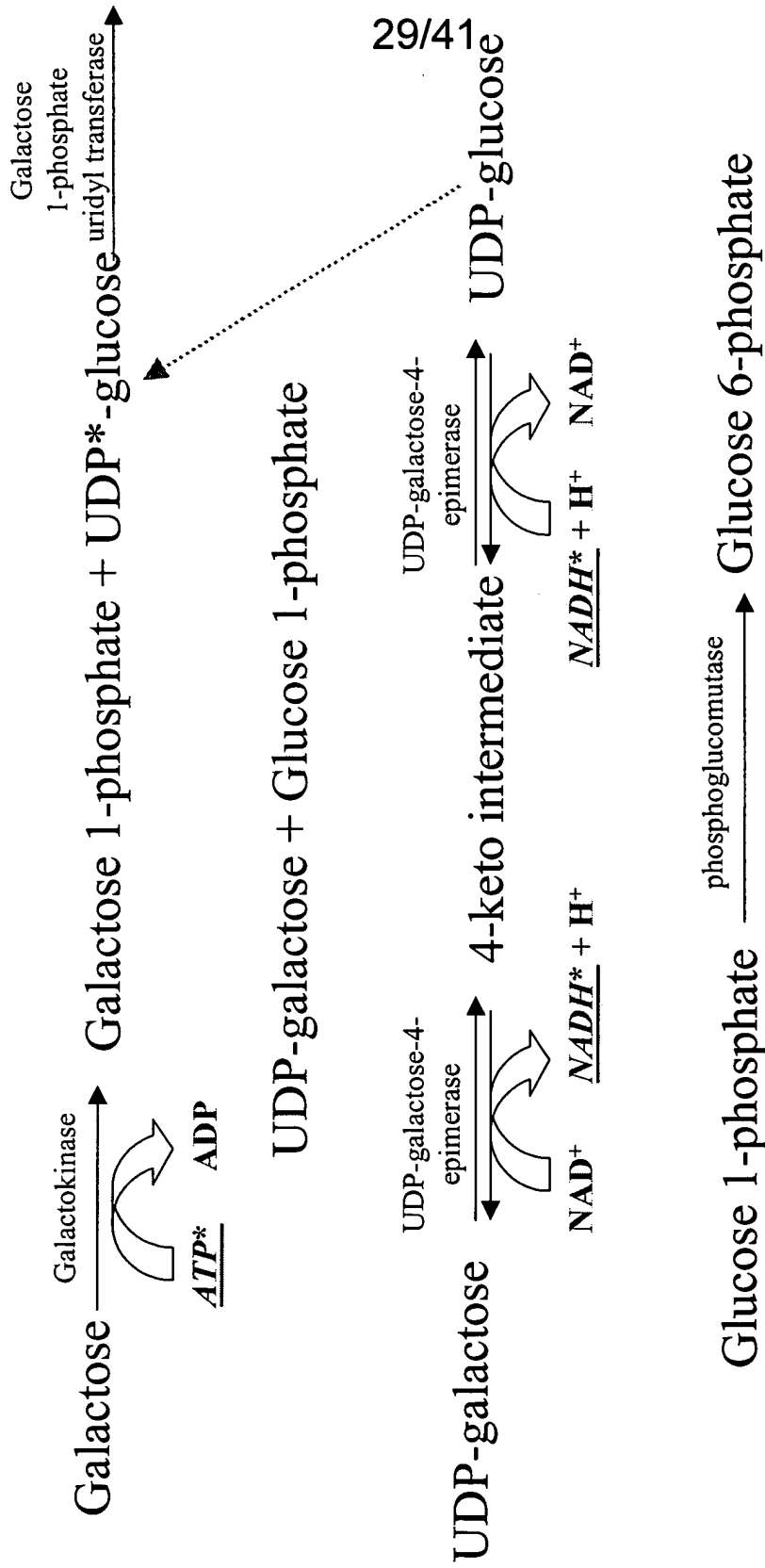
FIG. 25

FRUCTOSE GLYCOLYSIS



Continuation of Glycolysis

*uridine diphosphate (UDP)



Continuation of Glycolysis

*uridine diphosphate (UDP)

***Detectable Analytes (direct or indirect)** **FIG. 27**

GALACTOSE GLYCOLYSIS

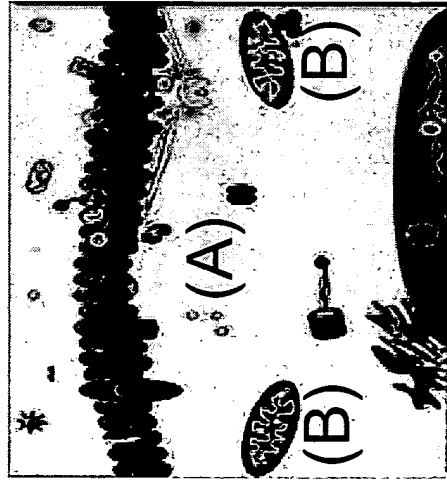
SMMR Mechanisms of Signal

- 1.0 Enhancement of Signal-to-noise of native autofluorescence
 - 1.1 Energy Transfer from NADH, NAD(P)H, or FAD to Reporters
(boosts signal by 5 to 50) indicating redox transfer coenzyme activity within cells and tissues
 - 1.2 Redox potential Reporters indicates number of mitochondrial transmembrane redox potential events
- 2.0 Enhancement of Specific Metabolite and Precursor Signals
 - 2.1 Lactate Reporters indicate lactate formation from anaerobic glycolysis activity
 - 2.2 Ca^{2+} Reporters indicate available ATP and ion pump transport activity fueled by glycolytic activity
- 3.0 Direct Glucose Reporters indicating quantitative levels of d-glucose
 - 3.1 Protein-labeled fluorophores
 - 3.2 proteins with a photooxidizable cofactor (such as FAD) to observe $^3\text{FAD}^*$

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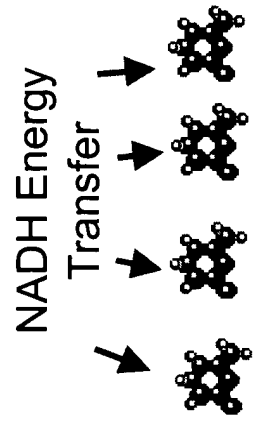
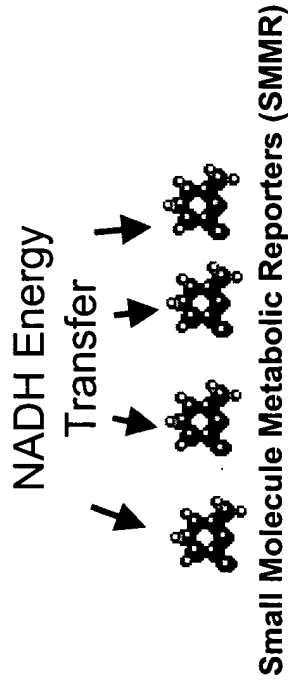
Energy Transfer Reporters

Glycolysis within the cell Cytosol (A)



Cell Cross-section

Within the Mitochondria (B)



Small Molecule Metabolic Reporters (SMMR)

FIG. 29

Redox Potential Reporters

In the Mitochondria



Increase in glucose concentration increases the mitochondrial membrane potential causing more small molecule metabolic reporter (SMMR) units to attach to the membrane. This causes fluorescence quenching proportional to changes in glucose concentration

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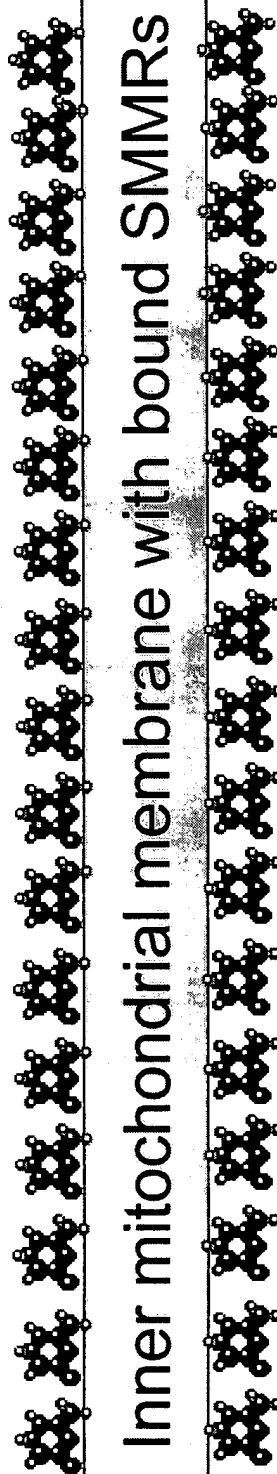


FIG. 30

Lactate Reporters

Anaerobic Glycolysis



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Increase in glucose concentration increases the lactate formation in a 2:1 ratio. A small molecule metabolic reporter (SMMR) is used to detect pH changes caused by lactate concentration. The pH changes are directly related to glucose concentration

pH change reduces FL of SMMRs



FIG. 31

Ca²⁺ Reporters

Ca²⁺ and ATPase

Cell signaling is accomplished using ions such as Ca²⁺. When the cell performs a signaling action Ca²⁺ is released from ion storage into the cytosol where it triggers cellular activities. A small molecule metabolic reporter (SMMR) is used to detect Ca²⁺ changes caused by changes in ion concentration within the cytosol. The Ca²⁺ concentration changes are directly related to healthy cell function. After signaling the Ca²⁺ is pumped back into storage using ATPase synthesized from Available ATP. Each molecule of ATP pumps 2 Ca²⁺. If the ion pumps are not working due to respiratory stress the ion concentrations equilibrate by diffusion since the pumps are incapacitated. The ion concentration gradients are maintained by ATP regulated pumps.

Ca²⁺ changes increase FL of SMMRs



FIG. 32

O₂ Reporters

Aerobic Respiration



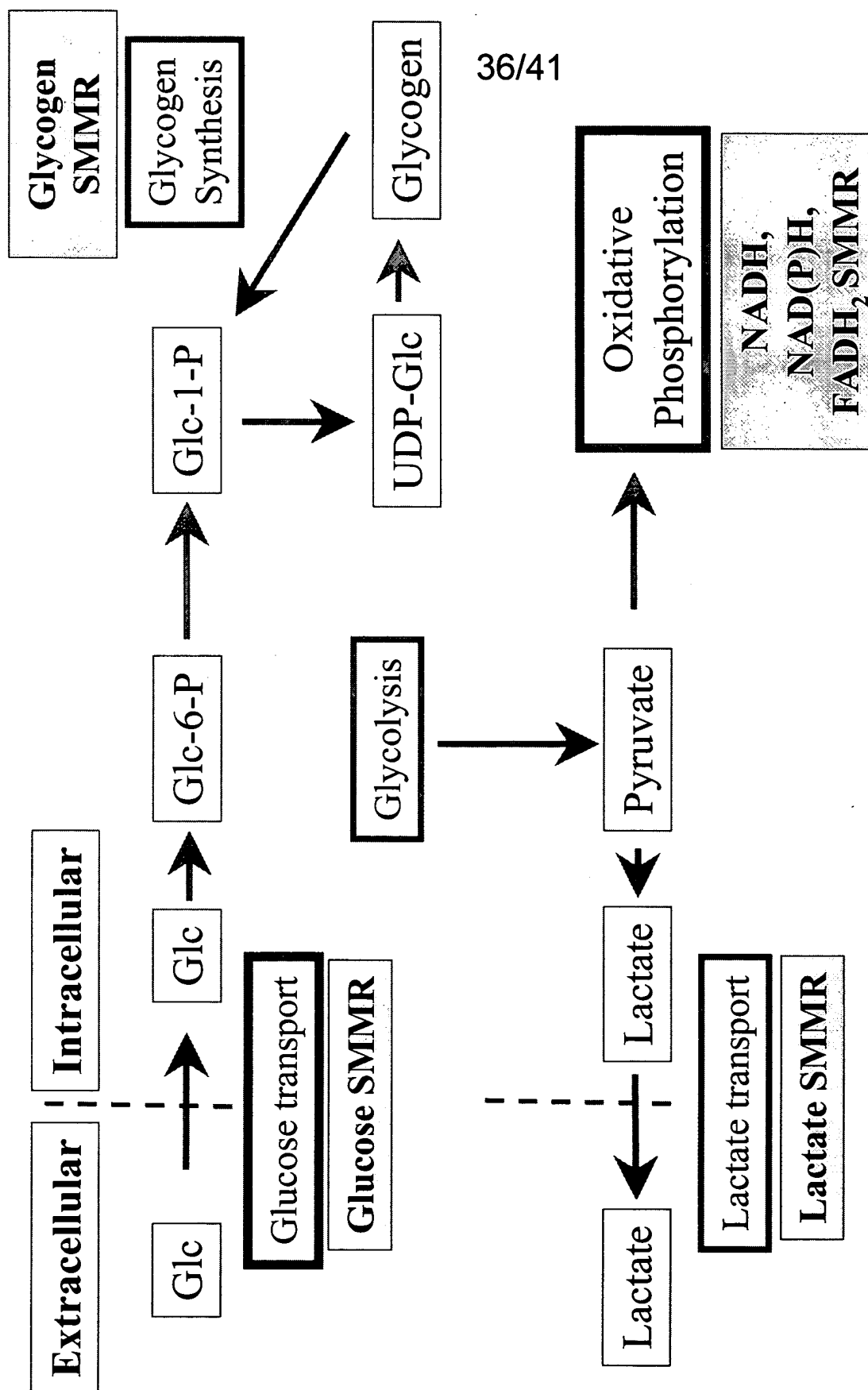
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Increase in molecular oxygen indicates a favorable environment for aerobic respiration. A small molecule metabolic reporter (SMMR) is used to detect O₂ changes in the cellular environment. The O₂ changes are directly related to ability to manufacture ATP.

O₂ changes increase FL of SMMRs



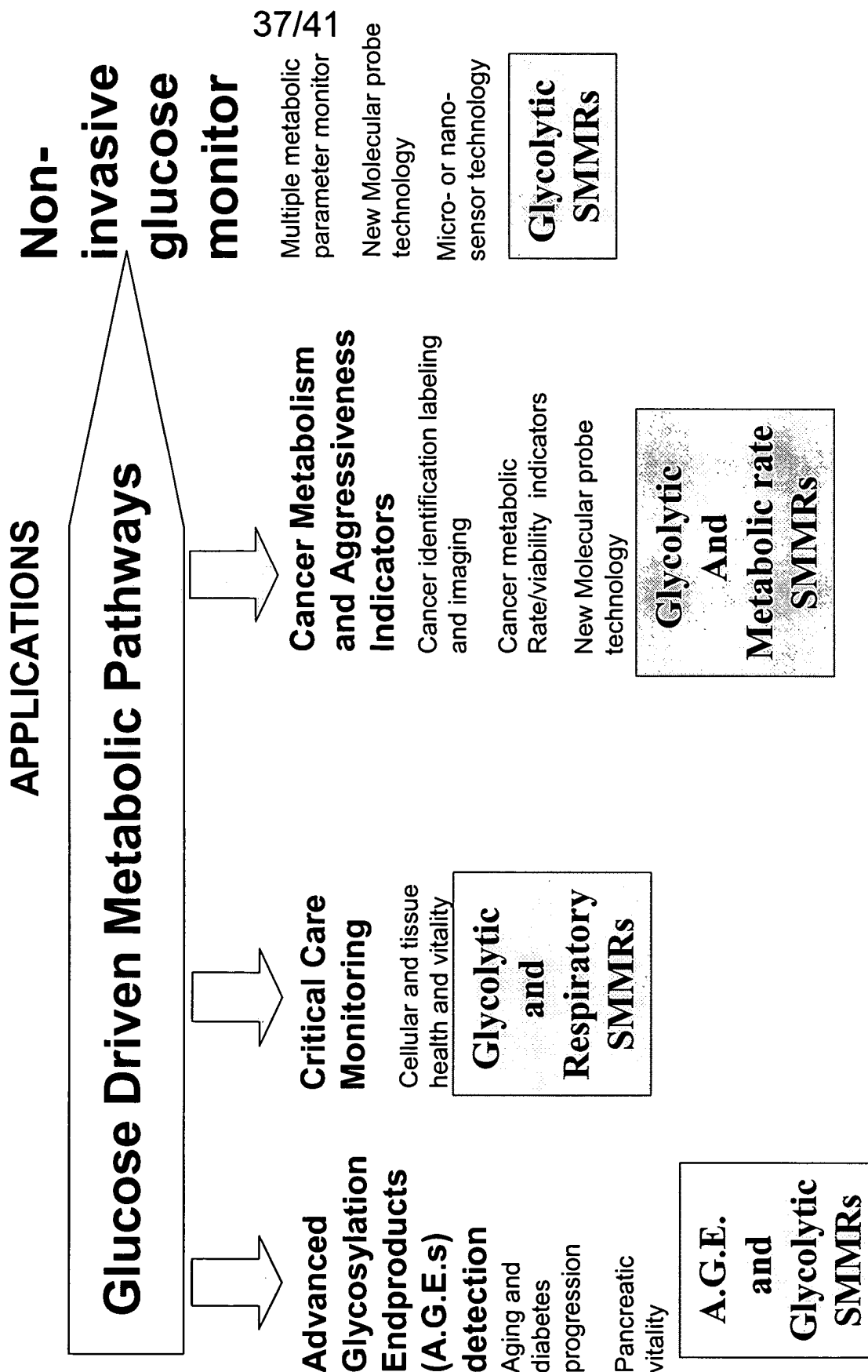
FIG. 33



SMMR has been used to establish analytical methods for measuring each glucose pathway for a variety of cell types

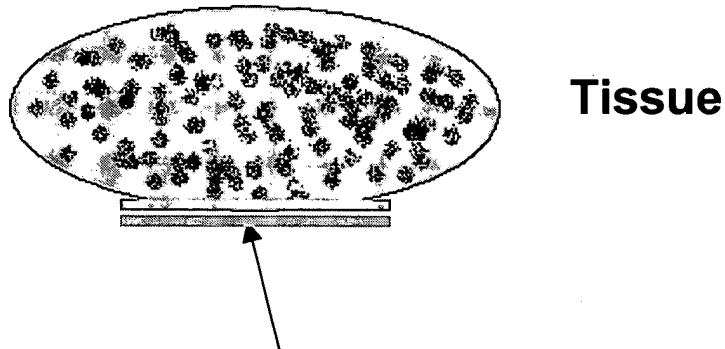
FIG. 34

SMMR Application Summary



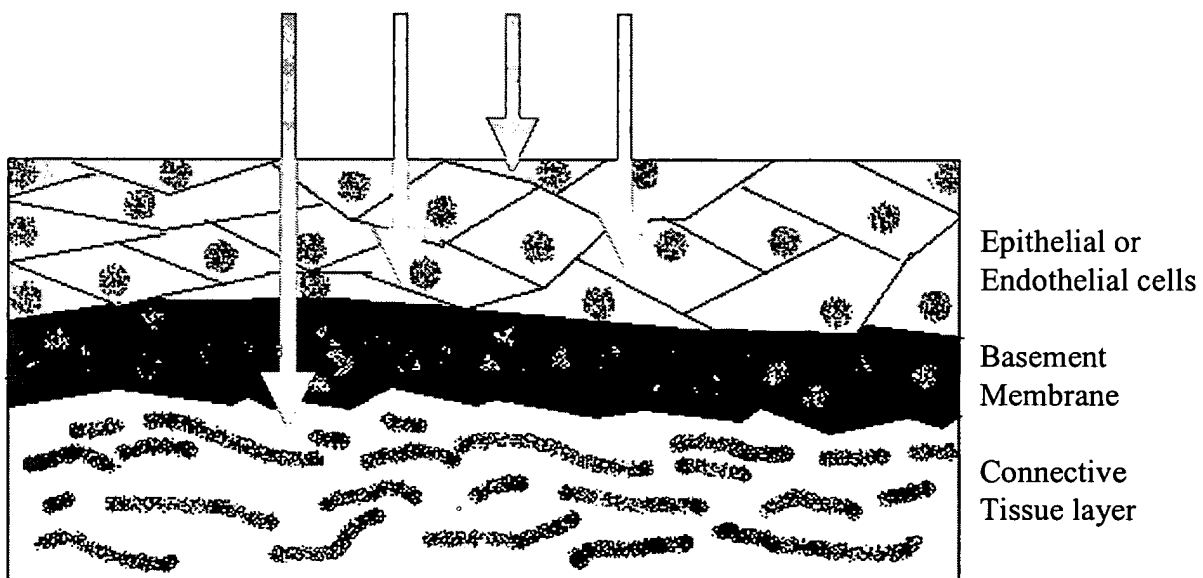
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Method for adding SMMR to peripheral epithelial cells in tissues and organs



A. SMMRs are applied to tissue surface

B. SMMRs are transported for up to 10-300 microns into the top of the tissue using passive or active transport



Outer (or inner) membrane of tissues and organs

FIG. 36

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SMMR for Metabolite Discrimination or Imaging

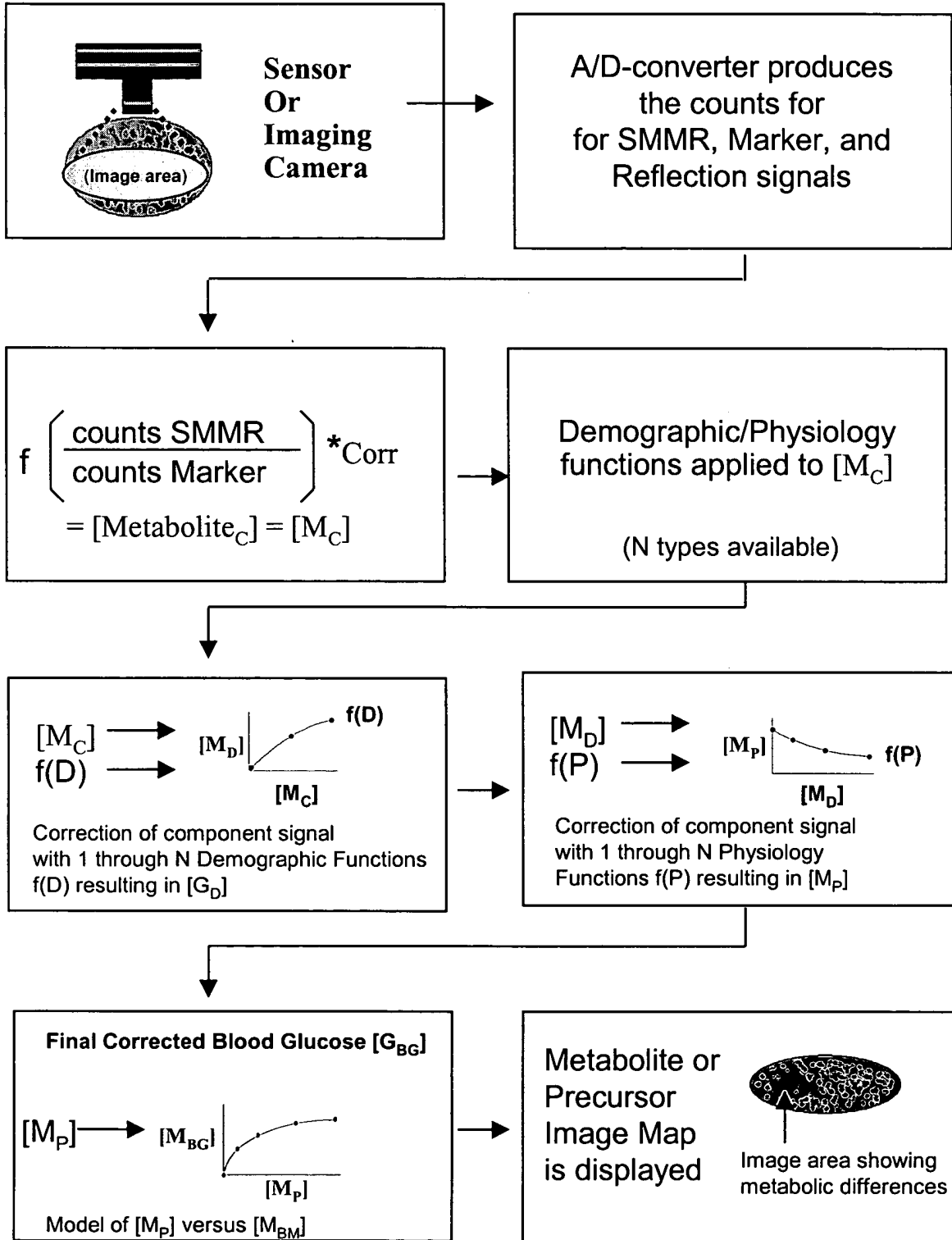


FIG. 37

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**CW Experiments - Fluorescent Response vs Glucose Addition
(Concs in mmol) - averaged data**

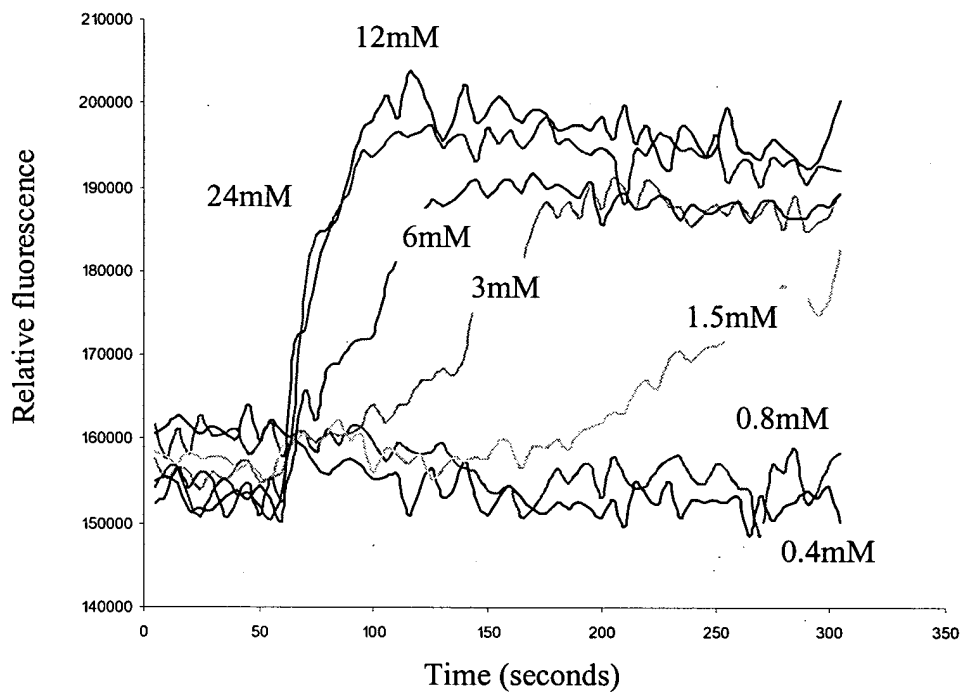
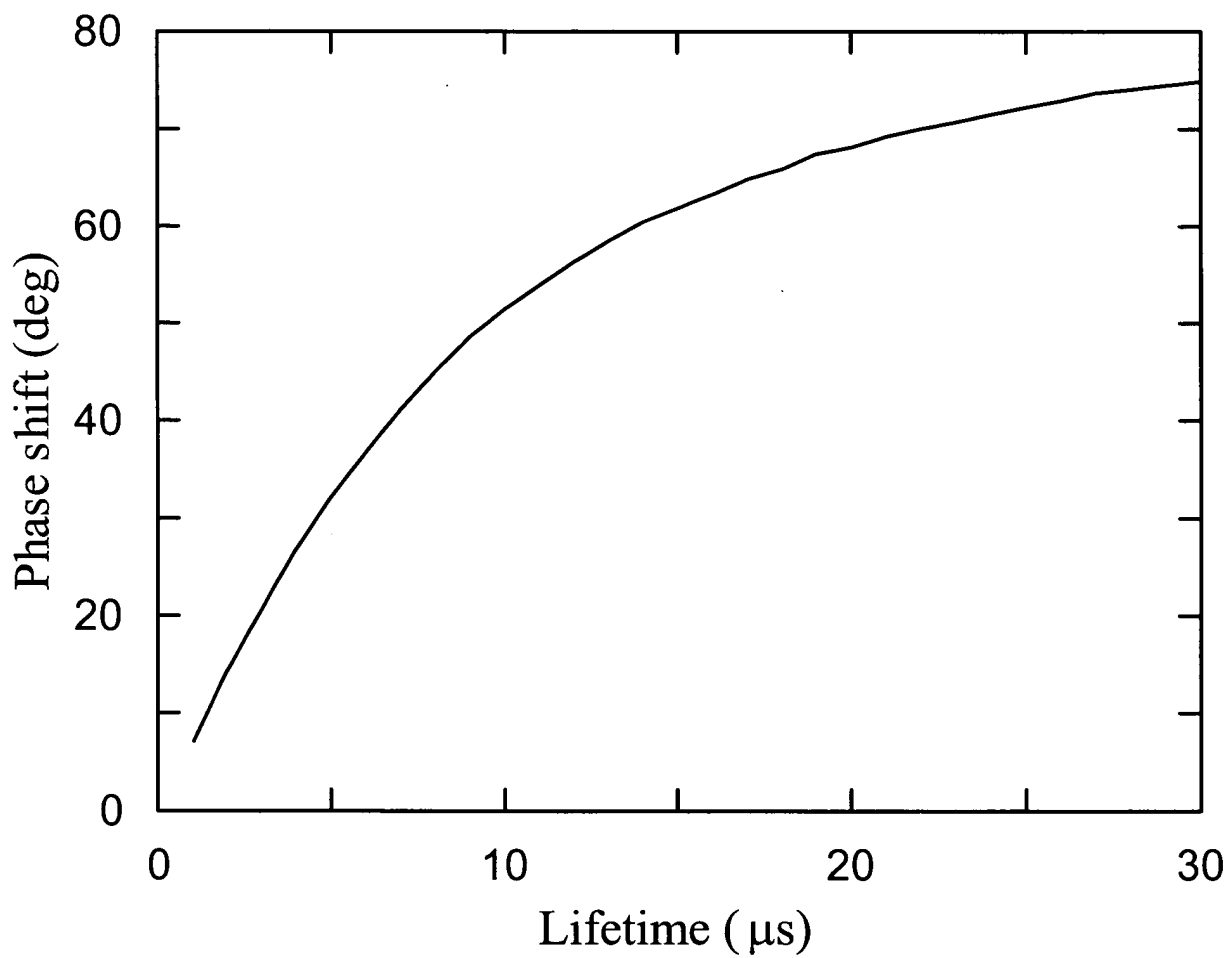


FIG. 38

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Plot of phase shift vs transient lifetime
with a modulation frequency of 2×10^4 Hz

**FIG. 39**